1990

# Effect of porcine somatotropin and stress susceptibility on the sensory, physical and chemical properties of porcine skeletal muscle

Jane Ann Boles
*Iowa State University*

# INFORMATION TO USERS

The most advanced technology has been used to photograph and reproduce this manuscript from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

**The quality of this reproduction is dependent upon the quality of the copy submitted.** Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313/761-4700 800/521-0600

Order Number 9100484

# Effect of porcine somatotropin and stress susceptibility on the sensory, physical and chemical properties of porcine skeletal muscle

Boles, Jane Ann, Ph.D.

Iowa State University, 1990

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

# Effect of porcine somatotropin and stress susceptibility on the sensory, physical and chemical properties of porcine skeletal muscle

by

Jane Ann Boles

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Departments: Food Technology
Animal Science
Major: Meat Science

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Signature was redacted for privacy.

For the Major Departments

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1990

# TABLE OF CONTENTS

page

INTRODUCTION ..... 1

LITERATURE REVIEW ..... 3

Skeletal Muscle Structure ..... 3

Myofibrillar/Cytoskeletal Proteins ..... 5

Calpain (Calcium Activated Factor) ..... 19

Skeletal Muscle Development ..... 21

Growth Hormone ..... 22

Porcine Stress Syndrome ..... 33

Water-Holding Capacity ..... 49

Sensory Evaluation ..... 52

Cookery ..... 54

SECTION I: EFFECT OF PORCINE SOMATOTROPIN, STRESS SUSCEPTIBILITY AND FINAL END POINT OF COOKERY ON THE SENSORY, PHYSICAL AND CHEMICAL PROPERTIES OF PORK LOIN CHOPS ..... 57

ABSTRACT ..... 59

INTRODUCTION ..... 60

MATERIALS AND METHODS ..... 62

Sensory Evaluation of Loin Chops ..... 63

Proximate Analysis ..... 65

Color Measurements ..... 65

Instron Measurement ..... 65

Statistics ..... 66

RESULTS AND DISCUSSION ..... 67
Acknowledgements ..... 75
REFERENCES ..... 76
SECTION II: PALATABILITY AND PROCESSING CHARACTERISTICS OF HAMS FROM PIGS OF THREE STRESS CLASSIFICATIONS TREATED WITH PORCINE SOMATOTROPIN ..... 80
ABSTRACT ..... 82
INTRODUCTION ..... 83
MATERIALS AND METHODS ..... 84
Ham Production ..... 85
Sensory Evaluation ..... 85
Proximate Analysis ..... 86
Color Measurements ..... 86
Statistics ..... 86
RESULTS AND DISCUSSION ..... 87
Acknowledgements ..... 91
REFERENCES ..... 92
SECTION III EFFECT OF PORCINE STRESS SYNDROME ON THE SOLUBILITY AND DEGRADATION OF MYOFIBRILLAR/CYTOSKELETAL PROTEINS ..... 95
ABSTRACT ..... 97
INTRODUCTION ..... 98
MATERIALS AND METHODS ..... 101
Myofibril Isolation ..... 101

Protein Solubility ..... 102
Whole Muscle Samples ..... 103
SDS Polyacrylamide Gel Electrophoresis ..... 104
Statistics ..... 105
RESULTS AND DISCUSSION ..... 106
Acknowledgements ..... 131
REFERENCES ..... 132
SUMMARY ..... 137
CONCLUSIONS ..... 141
REFERENCES ..... 143
ACKNOWLEDGEMENTS ..... 161

# LIST OF TABLES

page

SECTION I

Table 1: Mean sensory values of loin chops from placebo (control) and porcine somatotropin supplemented pigs ..... 68

Table 2: Mean sensory values of loin chops from three genotypes of stress susceptible pigs ..... 69

Table 3: Mean sensory values of loin chops broiled to two end-point temperatures ..... 70

Table 4: Mean values for proximate composition of loin chops from placebo (control) and porcine somatotropin supplemented pigs ..... 71

Table 5: Subclass means for lipid percentages of loin chops from placebo (control) and porcine somatotropin supplemented pigs of different stress susceptibilities ..... 72

Table 6: Mean Hunter Lab values for loin chops from PST treated pigs ..... 73

Table 7: Mean Hunter Lab values for loin chops from stress susceptible pigs ..... 73

Table 8: Mean percent cook yield of pork chops cooked to two end-point temperatures ..... 74

Table 9: Mean resistance to compression values of loin chops from animals of three classification of stress susceptible pigs ..... 75

SECTION II

Table 1: Mean sensory values for hams from animals of three genotypes of stress susceptible pigs ..... 88

Table 2: Mean values for proximate composition of hams from PST treated pigs ..... 89

Table 3: Mean values for proximate composition of hams from animals of three genotypes of stress susceptible pigs ..... 89

Table 4: Mean Hunter Lab values for hams from animals of three genotypes of stress susceptible pigs ..... 90

Table 5: Cooked yields for hams from animals of three genotypes of stress susceptible pigs.....90

## LIST OF FIGURES

page

SECTION III

Figure 1: Sarcoplasmic and myofibrillar protein solubility of *longissimus* muscle from animals of three stress classifications.....106

Figure 2: Effect of postmortem storage at 2°C on the solubility of protein from pork muscle from three stress classifications.....107

Figure 3: Ten percent SDS-PAG electrophoretogram of whole muscle homogenates and myofibrils (MF) from normal and stress positive (PSE) animals at 0, 3, and 7 days postmortem (S = Sigma high molecular weight standards) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa).....110

Figure 4: Five percent SDS-PAG electrophoretogram of myofibrils (MF) and whole muscle homogenates (WM) from normal and stress positive (PSE) animals at 0, 3, and 7 days postmortem (40 μg load per lane).....113

Figure 5: Ten percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 0 days postmortem from normal and PSE muscle (S=Sigma high molecular weight standard, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa).....116

Figure 6: Five percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 0 days postmortem from normal and PSE muscle (S=bovine titin, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg load per lane)..... 119

Figure 7: Ten percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 7 days postmortem from normal and PSE muscle (S=Sigma high molecular weight standards, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa)..... 122

Figure 8: Five percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 7 days postmortem from normal and PSE muscle (S= bovine titin, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg load per lane)..... 124

Figure 9: Ten percent SDS-PAG electrophoretogram of myofibrils isolated after 0, 1, 3, 5, and 7 days of postmortem storage at 2°C from normal and PSE animals (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa) ..... 127

Figure 10: Five percent SDS-PAG electrophoretogram of myofibrils isolated after 0, 1, 3, 5, and 7 days of postmortem storage at 2°C from normal and PSE animals (40 μg load per lane) ..... 129

# INTRODUCTION

Consumer demand for leaner meat products has led the pork industry to produce leaner animals. Pork producers have the resources to produce leaner pigs and are looking for ways to make the production of pork more efficient. Selection of faster growing, more efficient animals has been the traditional method for reducing fat and increasing profits for the producer. Using a combination of genetic selection and growth promotant supplementation could lead to more efficient lean meat production. Recent swine production research using growth promotants, such as growth hormone (somatotropin), report a reduction of lipid deposition and an increase in protein accretion as well as increased daily gains. Some researchers, however, have seen an increase in the incidence of pale, soft, exudative meat with the injection of exogenous pituitary growth hormone at a level of 100 μg $kg^{-1}$ $day^{-1}$ between the body weights of 30 and 60 kg with slaughter at 90 kg or administration starting at 60 kg body weight continuing for 31 days and slaughter following on day 32 (Solomon et al., 1989; Solomon et al., 1990). Pale, soft, exudative (PSE) muscle is usually associated with stress susceptible animals (Topel et al., 1975; Cheah et al., 1984). As a result of stress, PSE muscle displays a rapid postmortem pH decline (Eikelenboom and van den Bergh, 1973; Warriss, 1982), a reduction in water-holding capacity (Penney, 1977; Warriss, 1982) and decreased protein solubility (Bendall and Wismer-Pedersen, 1962; Sayre and Briskey, 1963). Consequently, this condition leads to an undesirable loss of functionality of the proteins in

processed products and large weight losses in fresh and processed products. Because of these problems along with the decreased fat, processors are concerned about the effect of porcine somatotropin on the processing and palatability characteristics of the muscle from these animals. The objective of this research was two-fold. One was to determine the effects of exogenous porcine somatotropin on the palatability, proximate composition, and lean color of pork loin chops and processed hams. The other was to determine the effects of stress susceptibility on the solubility and degradation of the sarcoplasmic and myofibrillar/cytoskeletal proteins.

# LITERATURE REVIEW

## Skeletal Muscle Structure

A review of skeletal muscle structure and its constituent myofibrillar proteins is important for an understanding of the effect of stress on protein solubility, water-holding capacity, and the structure of muscle. This review will deal exclusively with mammalian skeletal muscle.

Mammalian skeletal muscle fibers are surrounded and held in position by a series of continuous connective tissue sheaths. The whole muscle is surrounded by a heavy connective tissue layer called the epimysium. Branching from the epimysium to encircle muscle bundles is the perimysium. Surrounding the individual muscle fiber is the connective tissue layer called the endomysium. The cell membrane of the muscle cell, the sarcolemma, loosely encloses the cell and is closely associated with but separate from the endomysium (Forrest et al., 1975).

Skeletal muscle fibers are long, cylindrical in shape, multinucleate, mostly unbranched and striated. Muscle cells are multinucleated because they are formed by the fusion of mononucelated cells. The length of a muscle cell though variable averages from 20-30 mm and the fiber diameter, also variable, averages from 50-100 μm. Within the muscle cells are highly organized protein threads, the myofibrils, (Goll et al., 1984) the organelles responsible for contraction (Huxley, 1958).

Huxley (1958) reported that the arrangement of the protein filaments within the myofibril was responsible for the cross-striations observed with the phase contrast light microscope. The striated appearance is caused by precisely aligned dark and light bands of adjacent myofibrils. The dark bands of the myofibril are strongly birefringent (anisotropic) in the polarized light microscope, and are called the A-band. The light regions in the polarized light microscope are weakly birefringent (isotropic), and are called the I-band. Bisecting the I-band is a narrow dark zone called the Z-line. A less dense area exists in the center of each A-band and is known as the H-zone. A narrow band bisecting the H-zone is seen with the electron microscope and it is called the M-line. The sarcomere is the repeating structural unit of the myofibril and is the distance from Z-line to Z-line. The sarcomere length in resting muscle is 2.3-2.5 μm and in contracting muscle is 1.8-2.0 μm (Goll et al., 1984).

Huxley (1965) observed skeletal muscle by using the electron microscope and described the myofibrils as being composed of interdigitating thick and thin filaments. Thick filaments comprise the A-band and are 14-16 nm in diameter, 1.5 μm in length. The primary protein constituent is myosin, along with C-protein and the M-line associated proteins. Thin filaments compose the I-band and are 6-8 nm in diameter and 1 μm long. The major proteins making up thin filaments are actin, tropomyosin and troponin. One end of the thin filament is anchored in the Z-line and allows the tension developed in muscle contraction to be transmitted to adjacent sarcomeres.

Sarcomere length changes during contraction, but the lengths of the thick and thin filaments do not change (Huxley, 1965).

Goll and co-workers (1984) reported that adult mammalian skeletal muscle was 15-23% protein. These proteins are generally placed into three groups based on their solubility. Sarcoplasmic proteins are the most soluble, generally in water or very low ionic strength buffers, and are found in the cytoplasm. Of the three groups, the myofibrillar/cytoskeletal proteins are the largest fraction of proteins in the muscle cell. They are insoluble in water, but soluble in dilute salt solutions. The third group are the least soluble, called stromal or connective tissue proteins. Most of the proteins of this group are collagen and elastin.

Myofibrillar/cytoskeletal proteins compose 50-56% of total muscle proteins. A list of the more important myofibrillar proteins and their respective content are as follows: myosin, 45-53%; actin, 15-20%; tropomyosin, 4-6%; troponin, 4-6%; titin, 10%; nebulin, 5%; α-actinin, 2-3%; β-actinin, 1%; C-protein, 2%; M-protein, 3%; myomesin, <1%; creatine kinase, <1%; and desmin, <1% (Yates and Greaser, 1983; Goll et al., 1984).

## Myofibrillar/Cytoskeletal Proteins

Myosin comprises 94-96% (Goll et al., 1984) of the thick filament. It serves a very important biological function, because myosin along with actin are the contractile proteins of the myofibril. Myosin, molecular weight of 475,000, contains six subunits of two heavy chains and four light chains. The myosin molecule physically resembles a long

rod or tail with two pear-shaped heads at one end. Each head region contains two different light subunits, one 25 kDa or 16 kDa subunit and one 18 kDa subunit, and the tail contains the carboxy termini of two heavy chains. The globular head regions are the site of enzymatic and contractile activity. The entire molecule is 170-175 nm long with the rod portion being 155-160 nm long and 1.5 nm in diameter and the two pear-shaped heads being 18-19 nm long and 6-6.5 nm in diameter (Squire, 1986). Myosin binds to actin and other molecules, cleaves ATP enzymatically, and self assembles into thick filaments *in vitro*. The formation of thick filaments, results from the aggregation of the rod portion of adjacent myosin molecules forming the shaft of the thick filament, allowing the globular heads to extend outward and form the actin-binding cross-bridges (Squire, 1986).

Actin, a monomeric protein of 42,000 daltons, binds myosin and thereby modifies its $Mg^{2+}$-ATPase activity. Each molecule of actin contains one molecule of ATP and one $Ca^{2+}$ ion. In muscle cells, actin exists almost entirely in the filamentous multimers; two of which wrap together in a helix to form the backbone of the thin filaments. The $Ca^{2+}$ dependent interaction of myosin and actin is moderated by the tropomyosin/troponin complex which binds to actin (Pollard and Cooper, 1986).

Tropomyosin is a rod-shaped molecule 42.3 nm long. These rod-shaped molecules lie in the two grooves of the double-stranded actin filament, join end-to-end, and run the entire length of the actin filament (Goll et al., 1984). Tropomyosin is composed of two subunits,

α-tropomyosin and β-tropomyosin. The amino acids of both subunits are organized into heptad repeats that form the coiled coil structure of the molecule which permits a tight fit into the F-actin grooves (Mak et al., 1980). The amino terminus of the tropomyosin molecule is necessary for actin-tropomyosin interaction (Heald and Hitchcock-DeGregoris, 1988).

Troponin is a globular molecule, and is attached to tropomyosin at periodic intervals 38.5 nm apart along the thin filament. Tropomyosin and troponin together confer $Ca^{2+}$ regulation of contraction upon the muscle. Troponin is a complex molecule containing three different subunits, troponin-T (TN-T), troponin-I (TN-I), and troponin-C (TN-C). Troponin-T is the subunit that attaches the troponin complex to tropomyosin (Goll et al., 1984). It is required to produce $Ca^{2+}$ inhibition of ATPase activity (Zot and Potter, 1987). Troponin-T forms the long rod-shaped tail of the troponin complex (Zot and Potter, 1987). Troponin-I binds to both TN-T and TN-C and forms part of the globular head of the troponin-complex (Zot and Potter, 1987). It inhibits the actin-activated ATPase activity of myosin (Goll et al., 1984). This inhibition is not $Ca^{2+}$ dependent when the rest of the troponin-complex is absent (Zot and Potter, 1987). Troponin-C binds both to TN-T and TN-I but not to actin. Troponin-C has two high-affinity $Ca^{2+}$ binding sites per molecule. These sites bind $Ca^{2+}$ reversibly to supply the trigger for contraction and relaxation (Goll et al., 1984). One model for the control of contraction suggests that conformational changes in TN-T and TN-I caused by binding of $Ca^{2+}$ to

TN-C, shifts the tropomyosin further into the groove of the double-stranded actin filament exposing the actin to the myosin cross-bridges (Zot and Potter, 1987).

Immunofluorescence studies have shown that α-actinin is associated with actin and is the major protein located in the Z-line (Suzuki et al., 1976). Alpha-actinin is a homodimer composed of subunits 100,000 daltons each (Suzuki et al., 1976). The molecule constitutes 2-3% of the myofibrillar proteins and is 3.9 nm wide and 50 nm long (Suzuki et al., 1976). It has been proposed that α-actinin attaches thin filaments of opposing sarcomeres at the Z-line (Yamaguchi et al., 1985).

Desmin, first isolated from smooth muscle (Huiatt et al., 1980), and then from skeletal muscle (O'Shea et al., 1981), comprises the 10 nm diameter, "intermediate" filaments (Huiatt et al., 1980). Immunofluorescence localization studies suggest that desmin is located around the periphery of the Z-line (Robson et al., 1981) and may link adjacent myofibrils together at the Z-line (Huiatt et al., 1980; O'Shea et al., 1981). Desmin may be important in meat palatability because it disappears during postmortem aging (Robson et al., 1981).

C-protein is a constituent of the thick filament of the myofibril. It has a molecular weight of 140,000 (Starr and Offer, 1978) and it represents 2% of the total myofibrillar protein (Goll et al., 1984). There are seven bands, 43 nm apart, of C-protein on each side of the M-line on the thick filament (Craig and Offer, 1976). Various functions have been suggested for C-protein such as those affecting the actin-myosin

interaction, influencing thick filament length, modifying myosin cross-bridge movement and maintaining the thick filament shape during muscle contraction (Starr and Offer, 1978; Moos, 1981).

M-line proteins make up less than 3% of the myofibrillar proteins and are associated with the thick filament (Goll et al., 1984). The M-line of thick filaments is composed of three proteins, M-protein (165 kD protein), myomesin (185 kD protein) and creatine kinase (two subunits, 42 kD each) (Trinick and Lowey, 1977; Grove and Eppenberger, 1983). When longitudinal sections of muscle are examined by electron microscopy, the M-line appears as 3 to 5 lines perpendicular to the long axis of the thick filament. Trinick and Lowey (1977) proposed that the M-line functions to hold the thick filaments in their proper three-dimensional register.

Sjostrand (1962) stretched striated frog muscle and saw that the tapering ends of the thick A-band filaments appeared to be connected with the I-band network. When maximum stretch *in situ* was achieved, I-band filaments were pulled away from the A-band leaving a space of low opacity between the A- and I-band filaments with this space being crossed by very thin filaments. These filaments appeared to be continuous with A-band filaments and were thinner than I-band filaments. Considerable variation in thickness, and lack of 400 Å periodicity, also made them appear different from the I-band filaments (Sjostrand, 1962). dos Remedios and Gilmour (1978) later observed in glycerated rabbit psoas fibrils, extraction resistant filamentous material which joined the Z-disks. Locker and Leet (1975) stretched

beef *sternomandibularis* muscle and observed the changes with phase contrast microscopy and electron microscopy. Under the phase contrast microscope highly stretched fibers revealed well-spaced light and dark bands. In the electron microscope they saw that actin and myosin filaments no longer overlapped and had the same high degree of order as in a relaxed muscle. A light zone appeared at the A-I junction, but filaments still crossed the zone. They called these filaments, gap filaments. Since the identification of gap filaments, considerable efforts have been made to isolate an elastic protein which could be their primary constituent. In an investigation on filamin, Wang and co-workers (1979) observed 3 bands of high molecular weight proteins on low percentage SDS-PAG electrophoretograms. The largest of these proteins migrated as a closely spaced doublet on low percentage SDS-gels which they called titin-1 ($T_1$) and titin-2 ($T_2$). Thus from these data they suggested the existence of a third set of filaments comprised mainly of high molecular weight proteins supporting the existence of gap filaments. Their titin antiserum was specific for titin and had no cross-reactivity with actin, myosin nor filamin, demonstrating that this large protein called titin was antigenically distinct from myosin and filamin. Kimura and Maruyama (1983), independent of Wang (1982), purified a very large molecular weight protein that they called connectin which was similar to titin. Gap filaments which are made-up of titin (connectin), are slightly smaller in diameter than actin filaments, less numerous then actin filaments, and highly elastic (Locker et al., 1977).

Titin is a large, megadalton, insoluble molecule found in skeletal muscle (Maruyama et al., 1980; Wang et al., 1979; Trinick et al., 1984). Trinick and co-workers (1984) observed that it is soluble in 0.6 M KCl and that the solubility does not change by increasing ionic strength or time of extraction. They reasoned its insolublity was because the molecule was stabilized by covalent crosslinks. Circular dichromic analysis revealed that titin has a high proline content, and therefore its tertiary structure has a high percentage of random coil (Trinick et al., 1984). Titin is a highly asymmetric molecule having a long, string-like structure, with a beaded appearance which had a repeat distance between beads of 40 Å. The length seems to be variable (Trinick et al., 1984). Hainfeld et al. (1988) used scanning transmission electron microscopy (STEM) to determine the morphology and size of titin. They found two predominate types of morphology: one appeared as compact globules with stringy perimeters and dense cores roughly 25-35 nm in diameter; the other appeared as a loose ball of string that spread well over 80 nm. They determined the mass of $T_2$, the smaller more soluble band of the doublet, to be approximately $2.4 \pm 0.5 \times 10^6$ Da. This agrees with the average molecular weight of $2.4 \times 10^6$ and $2.6 \times 10^6$ determined by sedimentation equilibrium in guanidine hydrochloride, by Kurzban and Wang (1988). They determined from their data that the molecular weights represent a mixture of roughly equal amounts of $T_1$ and $T_2$ (Kurzban and Wang, 1988). Kurzban and Wang (1988) concluded from their studies that native $T_2$ is monomeric and that it is an extremely long, flexible and slender filament, and that

this reflects the specific folding of a single multimegadalton polypeptide. Wang et al. (1984) observed low-angle rotary-shadowed $T_2$ in the electron microscope and it appeared predominantly as very long, flexible, filamentous strands with irregularly spaced sharp bends and kinks. At higher magnification some strands had large globular nodules at either end that arose from coiling of ends. The shadowed width varied from 7-12 nm and the estimated width of the strand was 4-6 nm. The overall morphology of the fibrous network suggested that long titin strands must associate end-to-end as well as side-by-side to form long bundles of various sizes to form networks.

Locker and co-workers (1977) suggested that gap filaments formed a core in the thick filament, emerging at one end only, and passing between the thin filaments of the next sarcomere and into a second thick filament where it terminated as a core. Maruyama and colleagues (1980) suggested, based on antibody labeling, that connectin formed a net around the sarcomere, and Wang and co-workers (1984) and Wang and Wright (1988) believe titin makes-up a third set of elastic filaments.

Using fluorescence labeled-antibodies, Maruyama et al. (1980) localized connectin in the A-band and Z line, and found weak staining in the I-band. They concluded that connectin formed nets of very thin filaments (20 Å) around the sarcomere. Antibodies to titin and nebulin, however, did not stain smooth muscle (Furst et al., 1988). Furst and colleagues (1988) observed that each of 6 titin antibodies provided a pair of thin decoration lines per sarcomere. The position of

these 6 pairs was centrally symmetric to the M-line and changed in characteristic fashion with each antibody. Four other antibodies labeled distinct positions along the I band. These ten monoclonal antibodies to titin established a linear epitope map along the sarcomere. From their results, these researchers concluded that titin filaments clearly extend from the A-band and contact the Z-line. For each 1/2 sarcomere, the titin filaments are polar structures without epitope repeat (Furst et al., 1988). Whiting et al. (1989) also suggested that titin extends from Z-line to M-line. Wang and co-workers (1979) made titin antibodies that reacted with the myofibril in two specific regions, the junctions of A- and I-bands and the central region of the A-band, with staining being dependent upon sarcomere length. From this work they suggested that titin was a component of transverse structures such as M or Z lines (Wang et al., 1979).

The functions of titin are not proven, but are variously identified as participating in elasticity and scaffolding for development. Furthermore, titin filaments are thought to confer resting tension to muscle and the ordering of myofibrils. Horowits and co-workers (1989) observed that antibody labeling of connectin (titin) of myofibrils at different stages of contraction were at different places. In relaxed fibers, antibody binding is visualized as two extra striations per sarcomere arranged symmetrically about the M-line. These striations move away from both the nearest Z-line and the thick filaments when the sarcomere is stretched, confirming the elastic behavior of connectin within the I-band of relaxed sarcomeres.

Relaxed fibers exhibit sarcomeres of uniform length with centrally located A-band. Resting tension is the force that is exerted to maintain the A-band in the middle of the sarcomere. Resting tension declines immediately after stretch, but is relatively stable after five minutes. Resting tension is undetectably small at lengths less than 2.5 μm, and between 2.6 and 2.8 μm the resting tension-length relation, curves upward and becomes linear at higher sarcomere lengths. The active tension-length relation decreases linearly in unstretched fibers as overlap between thick and thin filaments is reduced from a maximum to zero. A-band movement is significantly slower than tension development. The lag between maximum tension development and the completion of A-band movement may account for the fact that this phenomenon is not always apparent. These data strongly support the hypothesis that the elastic titin filaments produce most of the resting tension and tend to stabilize the position of the thick filaments at the center of the sarcomere during contraction (Horowits and Podolsky, 1987).

In a similar vein, Horowits and co-workers (1986) irradiated skeletal muscle and saw a decrease of titin and nebulin intensity on gels. Irradiation also decreased resting tension at sarcomere lengths of 2.6 μm. They found after irradiation that the edges of the A-band were no longer as sharply defined and this was accompanied by a disordering of the M-line at the center of the sarcomere, indicating to these workers that there was a misalignment of the thick filament.

The results of these studies agree with the proposed function of titin in generating resting tension and ordering of the sarcomere.

Titin has been proposed to form filaments that extend from Z-line to Z-line, and because of this it has been suggested that it could form a scaffolding for development. Tokuyasu and Maher (1987) observed aggregates of several titin molecules in large numbers in cardiac myocytes before the formation of the first myofibrils. At the 5 somite stage titin labeling was found to occur diffusely throughout the cytoplasm, and at the 9 somite stage titin labeling was found to be confined to the heart trough, and at the 11 somite stage, the majority of titin spots were found as parts of myofibrils. These results confirm the presence of titin before other myofibrillar proteins in development, and support the suggestion that titin forms a scaffolding for development. Furthermore, Murayama and co-workers (1989) showed that purified myosin will bind to purified connectin (titin) but did not significantly change the length distribution of myosin filaments when added during filament formation, *in vitro*. These effects are independent of and not affected by C-protein. Titin, therefore, might create a scaffolding for the formation of the myofibril, but does not effect the formation of the individual components of the sarcomere.

Meat scientists are interested in the postmortem changes of myofibrillar/cytoskeletal proteins because of their potential effects on tenderness and water-holding capacity. Work on connectin showed that $T_1$ decreased during postmortem aging when observed on SDS-PAGE, and the degradation varied among muscles (Takahashi and Saito,

1979). Takahashi and Saito (1979), using anti-titin antibodies, also observed that connectin disappeared from myofibrils upon aging and saw a concomitant disappearance of connectin from SDS-gels. They concluded that the net around myofibrils was made of connectin. These postmortem changes observed were dependent on the presence of $Ca^{2+}$ (Takahashi and Saito, 1979). King and Kurth (1980) purified connectin and observed changes due to various aging conditions. *In vitro*, the extent of breakdown of connectin after heating at 60°C for 20 minutes was greater at a lower pH than at a higher pH and concluded that pH decline rather than other factors is a prime factor leading to the breakdown of connectin with time postmortem and that acid cathepsin may contribute to the breakdown of connectin during cooking at 60°C. Ringkob et al. (1988) using immuno-stained myofibrils from 48 hr postmortem muscle observed a decreased staining pattern from myofibrils isolated at death. Lusby et al. (1983) observed a disappearance of titin from SDS-PAGE with increasing storage time and temperature. Olson et al. (1977) reported an increase in fragmentation of myofibrils with postmortem aging. Myofibril fragmentation, the breakage of myofibrils into smaller number of sarcomeres at or near the Z-line, accounts for about 50% of the variation in beef steak tenderness (Olson and Parrish, 1977) Titin's disappearance upon aging and concomitant increase in fragmentation suggest that titin may have an effect upon tenderness. Calpain (calcium activated factor, CAF) the natural protease found in skeletal muscle weakens Z-disks causing an increase in myofibril fragmentation

(Olson et al., 1977). Because titin degradation is calcium dependent and because calpain's role is important in increasing tenderness with postmortem aging (Takahashi and Saito, 1979), tenderness may be the result of calpain degrading titin. Anderson and Parrish (1989) and Paterson and Parrish (1986) found a direct relation between tenderness and titin degradation as assayed by SDS-PAGE. That is, less tender steaks had less degradation of titin than their tender steak counterparts. Moreover, gap filaments remain after cooking in non-aged beef; but, in aged meat they disappear on cooking leaving gaps in the myofibril (Locker et al., 1977).

Titin may influence water-holding capacity of meat. Offer and Trinick (1983) presented evidence showing the removal of transverse structural constraints, Z- and M-lines and myosin cross-bridges, positively influenced water-holding capacity. Paterson and co-workers (1988) showed an increased extraction of titin concomitant with increased water-holding capacity associated with the addition of sodium chloride and pyrophosphate solutions. They also observed swelling of myofibrils with the addition of sodium chloride and pyrophosphates suggesting the removal of transverse structural constraints. From their results, these researchers concluded that titin makes up part of the structural constraints that influence water-holding capacity.

Nebulin with a molecular weight of 500,000 constitutes approximately 5% of the myofibrillar/cytoskeletal proteins (Goll et al., 1984). Like titin, nebulin has a high proline content (5.9%), but nebulin

has a higher total content of charged amino acids, lysine, arginine, aspartic acid and glutamic acid (41.1% vs 33.9%) (Wang and Wright, 1988). Larger nebulin-like proteins have been found in heart, uterus and gizzard; however, they do not cross-react with antibodies to skeletal muscle nebulin (Wang and Wright, 1988). The location of nebulin in the sarcomere has been studied using antibodies to nebulin. All sarcomeres within the myofibrillar bundle are uniformly labeled by nebulin antibodies to give rise to two transverse stripes within the I-band. Nebulin constitutes a set of discontinuous parallel and inextensible filaments attatched to the Z-line; there are 3 or 4 molecules per thin filament. All sarcomeres are uniformly labelled by the antibody and stained areas appear as transverse stripes. These stripes are the result of the labeling of epitopes that are aligned axially. Moreover, these epitopes are resistant to stretch which contrasts sharply with the elastic stretch-dependence of the coexisting I-band domain of titin filaments. These distinct behaviors indicate that nebulin and titin must each represent a separate set of parallel filaments that cannot be serially connected as components of the same longitudinal filaments.

Nebulin also may have a role in tenderness. It is susceptible to postmortem degradation and this degradation is time and temperature dependent (Lusby et al., 1983; Paxhia and Parrish, 1988). Zeece and co-workers (1986) reported that CAF (calpain) degraded nebulin, *in vitro*. Paxhia and Parrish (1988) reported that pork nebulin disappeared from SDS-gels after only 1 day of postmortem aging (4°C)

of muscle. Paterson and Parrish (1986) observed more nebulin in tough than in tender chuck muscles. Furthermore, Anderson and Parrish (1989) reported a more rapid degradation of nebulin in tender steaks (*longissimus*) than in their less tender counterparts. Supporting that nebulin may affect tenderness.

Calpain (Calcium Activated Factor)

Calpain is a calcium-dependent cysteine proteinase widely distributed throughout the body of animals, that displays limited proteolysis at neutral pH (Goll et al., 1985; Murachi, 1990). This proteinase is referred to by a variety of names. The proteinase has been termed CASF (calcium-activated sarcoplasmic factor), CANP (calcium-activated neutral protease), CAF (calcium-activated factor), and CAP (calcium-dependent protease) (Goll et al., 1985). Calpastatin, a specific inhibitor of calpain, is also a widely distributed intracellular component (Shannon and Goll, 1985; Murachi, 1990). Calpastatin is only effective on calpain and calcium is required for inhibition (Shannon and Goll, 1985; Murachi, 1990). Furthermore, two forms of calpain are known, Calpain I and Calpain II (Kishimoto et al., 1981; Wheelock, 1982; Goll et al., 1985; Murachi, 1990). These two forms require different levels of calcium for activation. Calpain I requires μmolar quantities of calcium for activation, while Calpain II requires millimolar quantities of calcium for activation.

Calpain possesses two polypeptide chains per molecule with masses of 80 kDa and 28 kDa (Suzuki et al., 1981; Goll et al., 1985; Murachi, 1990). The proteinase rapidly autolyzes in the presence of

calcium. The 80 kDa peptide is degraded to a 74-76 kDa peptide depending on the source of proteinase and whether it is the micro- or millimolar form, and the 20 kDa peptide is degraded to an 18 kDa peptide (Goll et al., 1985). This autolysis reduces the calcium requirement of both forms of calpain (I and II) (Goll et al., 1985; Murachi, 1990).

Antibody localization studies of calpain in myofibrils showed that it was localized on the Z-disk (Ishiura et al., 1980; Goll et al., 1985). When sections of bovine muscle were used, however, calpain was distributed throughout the muscle cell without preferential localization at the sarcolemma or the Z-line (Goll et al., 1985). Goll and co-workers (1985) concluded that the absence of high concentrations of calpain at the Z-line in fixed muscle sections, indicated either that the moderately hydrophobic calpain molecule is absorbed to this region during myofibril isolation or only a small portion of the total cellular calpain is bound to the Z-line but this calpain is only evident once the large amounts of calpain in the surrounding I-band are washed away during myofibril isolation.

Calpain activity and content are different in different muscles. Calpain activity is high in the *longissimus dorsi* and *biceps femoris* while it is low in the *psoas major* muscle (Koohmaraie et al., 1988a). Koohmaraie and co-workers (1988a) observed an increase in tenderness with postmortem aging in muscles that possessed high calpain activity. Koohmaraie and colleagues (1988b), also observed that the changes associated with increased postmortem tenderization

were arrested when EDTA and EGTA were added to the muscle. This suggested that changes were associated with divalent cation activation. In support of this, infusion of lamb carcasses with calcium chloride resulted in accelerated postmortem tenderization (Koohmaraie et al., 1988c). This suggested that the changes observed during postmortem storage appear to be associated with calpain activity.

## Skeletal Muscle Development

Skeletal muscle arises from the mesodermal layer of the embryo. Allen and co-workers (1975, 1979) arbitrarily divided myogensis into at least two general kinds of events: those events initiating the bulk synthesis of muscle specific proteins, and those events that control the mitotic activity and fusion of myogenic cells. The processes responsible for these two occurrences are cell proliferation and differentiation. During proliferation daughter cells are identical, at some point daughter cells are altered to be different from the mother cells, and this cell line that becomes muscle are called presumptive myoblasts. Presumptive myoblasts are mononucleated, and do not produce muscle specific proteins. The cells in the next stage of differentiation are myoblasts. Myoblasts are mononucleated and the synthesis of muscle specific proteins begins at this stage. Myoblasts can fuse to form myotubes. Myotubes are multinucleated, the nuclei are unable to divide and the synthesis and assembly of muscle specific proteins occurs. The final stage is the fusion of myotubes with other myotubes or with myoblasts to form muscle fibers. Only mononucleated cells have the ability to proliferate, the nuclei in myotubes cannot replicate

their DNA and divide. Therefore, the transition from proliferating myoblasts to myotubes that can synthesize muscle specific proteins represents the terminal step in differentiation (Allen, 1988). The number of muscle fibers present is established near the time of birth. Postnatal muscle growth is frequently considered to be primarily due to muscle hypertrophy, increased fiber size (Allen et al., 1979). Protein to DNA ratios, however, suggest that myogenic proliferation, hyperplasia, does not cease at birth (Allen et al., 1979). One explanation for postnatal hyperplasia is satellite cells. Sattelite cells are mononucelated cells located under the basement membrane that can divide and fuse with existing muscle to increase nuclei number (Allen et al., 1979). Satellite cell fusion to cause postnatal growth of muscle is under genetic and endocrine control (Martin and Ezekwe, 1975).

## Growth Hormone

Control of growth in animals is complex and involves a variety of hormones including thyroid hormones, growth hormone (somatotropin), somatomedins and other circulating growth factors, such as androgens, insulin, prolactin and glucocorticoids. Growth hormone (GH) is a naturally occurring protein hormone, its absence results in little or limited growth. In mammals and birds, growth hormone secretion is controlled by both stimulatory and inhibitory hypothalmic factors (Scanes and Lauterio, 1984), that is, growth-hormone-releasing factor and somatostatin help regulate the release of growth hormone from the pituitary gland. Growth hormone, released from the pituitary, acts on

the liver to cause the release of somatomedins. It is generally believed that the effect of growth hormone on muscle growth is mediated through somatomedins produced in response to growth hormone (Dayton, 1988). Somatomedins (Insulin-like growth factors, IGFs) are small polypeptides found in the serum (Dayton, 1988). Two classes of IGFs have been characterized: IGF-I or somatomedin c (IGF-I/SMC) and IGF-II or neutral somatomedin (IGF-II/NSM) (Dayton, 1988). IGF-I/SMC can stimulate growth in hypophyscetomized rats, proliferation and differention in cultured myoblasts, and amino acid uptake in cultured myoblasts (Dayton, 1988). IGF-II/NSM has also been shown to stimulate proliferation of cultured myoblasts and can stimulate protein synthesis in cultured myotubes (Dayton, 1988). From this, it appears likely that somatomedins, controlled by growth hormone, are potent stimulators of muscle growth and development (Dayton, 1988).

Administration of epinephrine, norepinephrine and some adrenergic agonists depress growth hormone levels in plasma (Scanes and Lauterio, 1984). Growth hormone affects metabolism of lipids, carbohydrates and proteins. In particular, growth hormone seems to influence lipid metabolism by increasing lipolysis, decreasing lipogenesis and also increasing glucose uptake by adipose tissues (Scanes and Lauterio, 1984). Growth hormone also appears to reduce fatty acids available for triglyceride synthesis (Scanes and Lauterio, 1984) leading to a decrease in triglycerides stored. This has led to the experimental use of exogenous growth hormone to increase muscle growth and decrease fat deposition.

In animals exogenous injections of GH and growth hormone releasing factor (GRF) have both been used experimentally to reduce fat deposition. Several researchers have observed an increase in average daily gain and feed efficiency (Baile et al., 1983; Chung et al., 1985; Etherton et al., 1986; Etherton et al., 1987; Bechtel et al., 1988; Ender et al., 1989a) by using growth hormone supplementation. Carcass composition has also been improved by the use of GH. Many researchers have reported a decrease in carcass lipid with a concomitant increase in protein and moisture (Etherton et al., 1986; Etherton et al., 1987; Bechtel et al., 1988; Beerman et al., 1988a; Ender et al., 1989b; Smith et al., 1989). Etherton and co-workers (1987) observed that the decrease in fat was not reflected by a significant decrease in backfat. Changes observed in carcass composition and feed efficiency were highly correlated with dose administered of both GH and GRF (Baile et al., 1983; Etherton et al., 1986; Etherton et al., 1987; Bechtel et al., 1988; Beerman et al., 1988b; Ender et al., 1989a,b). Considerable animal to animal variation has been observed with GH treatment (Etherton et al., 1986). Etherton and co-workers (1986) observed that porcine growth hormone increased growth, and improved feed efficiency 19%, decreased carcass lipid 18% and significantly increased carcass protein.

Baile and co-workers (1983) treated pigs with human growth hormone (hGH) at .015 mg $kg^{-1}$ $day^{-1}$ and .06 mg $kg^{-1}$ $day^{-1}$. The lower dose resulted in increased average daily gain but had no effect on feed efficiency; whereas the higher dosage had no effect on weight gain,

feed intake or feed efficiency. In addition, dressing percentages were lowered, carcasses were longer and trimmed belly weights were lower in GH treated groups; however, no effect on specific gravity, lean percentage, backfat thickness, loin eye area and percent ham and loin were observed (Baile et al., 1983). Chung and co-workers (1985) injected pigs starting at 32 kg with .022 mg $kg^{-1}day^{-1}$ of pituitary growth hormone. The administration of GH increased growth rate approximately 10% and improved feed efficiency. A 50% increase in intramuscular lipid in the *longissimus dorsi* muscle was observed, but no change in backfat or percent fat were noted (Chung et al., 1985). Furthermore, no deleterious effects on animal health was observed with administration of growth hormone, nor did it affect kidney and liver morphology or femur weight and length (Chung et al., 1985).

Peters (1986) showed a different response in growing beef steers to growth hormone when the steers were limited fed as opposed to full fed. Growth hormone (20 mg/d) did not affect final weight gain among *ad libitum* fed steers but treated steers tended to have a lower total feed intake. Furthermore, backfat, rib eye area, and protein content were increased in response to *ad libitum* feeding whereas growth hormone treatment resulted in lowered backfat, no effect on rib eye size and greater total protein content.

Ender and co-workers (1989a, b) treated Landrace barrows with 2 or 4 mg $hd^{-1}$ $d^{-1}$ of recombinant porcine somatotropin (PST), and reported increased carcass weights, as well as heavier heart, liver and kidney weights. Dressing percentages were decreased at both dose

levels and percentage of primary cuts was increased over controls by increased PST dose; back fat layer and lipid content were decreased with PST treatment; and PST enhanced growth performance, feed efficiency and net protein gain to the debit of lipid (Ender et al., 1989b). Similarly, Bechtel and co-workers (1988) treated pigs with 1.5, 3.0, 6.0 or 9.0 mg $hd^{-1}$ $d^{-1}$ of pituitary porcine somatotropin, and reported a dose dependent decrease in feed consumption, dressing percentage and an increase in average daily gain. In addition, backfat was decreased in a dose dependent manner. Beerman and co-workers (1988b) found the optimal dose for average daily gain to be 60 $\mu g$ $kg^{-1}$, for feed efficiency to be 120 $\mu g$, and for protein increase (56%) and lipid reduction (88%) to be 200 $\mu g$. Bark and co-workers (1989) treated animals of lean and obese phenotypes and a simliar decrease in fat and increase in growth in response of PST treatment was observed. There was a decrease in fat and an increase in growth for both genotypes. Smith and co-workers (1989) observed that recombinant PST improved growth, feed efficiency and carcass characteristics, and that the effect waned 10 days after the cessation of PST treatment.

The administration of exogenous GH can alter the serum profile of hormones, fatty acids and nitrogen. Injection of exogenous GH leads to an increase in plasma GH (Baile et al., 1983; Eisemann et al., 1986), increases in plasma insulin (Chung et al., 1985; Eisemann et al., 1986; Peters, 1986; Etherton et al., 1986), increases in plasma free fatty acids (Chung et al., 1985; Peters, 1986; Dunshea et al., 1989), and decreases in blood urea nitrogen (BUN) (Chung et al., 1985). Eisemann and co-

workers (1986) injected Hereford heifers with bovine GH (bGH), and observed its effects on hormones and metabolites. They found that daily injections of growth hormone resulted in a ten-fold increase in peak concentration of plasma growth hormone, and a nine-fold average increase during the 7 h post injection period. Peak concentration was approximately 2 h following injection indicating slow absorption of bovine growth hormone from subcutaneous injection sites. Moreover, plasma concentrations of growth hormone in bovine growth hormone injected heifers were similar to those in placebo-injected heifers by 24 h postinjection. Growth hormone injection caused no changes in circulating concentrations of prolactin, $T_3$, $T_4$, urea nitrogen, glucose or β-hydroxybutyrate; however, plasma insulin was chronically increased. From these results they concluded that growth hormone affects metabolism by altering steady-state set points within the animal to support growth, and markedly improves the efficiency of protein deposition. In addition, plasma non-esterified fatty acid concentration was chronically elevated, and no acute fluctuations were observed. Both irreversible loss of non-esterified fatty acids and oxidation of non-esterified fatty acids were higher in response to bovine growth. hormone treatment. Non-esterified fatty acid flux through the plasma pool was also observed. This suggested to the researchers an increase in lipolysis in response to bovine growth hormone. From this data, Eisemann and co-workers (1986) concluded that bovine growth hormone coordinates metabolism of several body tissues to enhance the process of lean body growth.

Baile and co-workers (1983) injected pigs with bacterially-synthesized human growth hormone (hGH) and observed plasma concentrations of human growth hormone. For all groups hGH concentrations were maximal one hour after injection, remained elevated for 2 hours, and gradually decreased up to 8 hours post injection, with it still being higher than at time zero. After 22 hours, serum hGH returned to base line. Greater injection concentrations led to greater serum concentration. The first injection of hGH stimulated insulin secretion in pigs, while after chronic injection of hGH, plasma insulin concentrations were decreased compared with those of control pigs. Furthermore, glucagon concentrations were lowered in pigs after the first injection of hGH, but were higher in pigs after chronic injection. Dunshea and co-workers (1989) also injected pigs with GH and observed its effects on carbohydrate and lipid metabolism. Somatotropin treatment significantly decreased $^{14}C$ labelled glucose incorporation into both triglyceride glycerol and fatty acids. The greater reduction in triglyceride fatty acids relative to glycerol synthesis under basal conditions is indicative of virtual reliance on preformed fatty acids for limited adipose tissue triglyceride synthesis in PST treated pigs. Glucose incorporation into lipid was reduced during hyperinsulinemia in the PST treated pigs whereas glucose irreversible loss rate was unchanged, possibly due to increased muscle and or hepatic glycogenesis. Etherton et al. (1986) observed that chronic treatment with GRF or GH increased serum glucose and was associated with an increase in serum insulin. This suggested to them

that target-tissue sensitivity was decreased by the elevation in serum GH. They also observed that adipose tissue responsiveness to GH changed during growth. Chung and co-workers (1985) injected pigs starting at 32 kg with 22 $\mu g\ kg^{-1}day^{-1}$ of pituitary growth hormone. They observed elevated plasma porcine growth hormone (pGH) levels of 10-13 fold within one hour of injection and elevation was maintained for 4-5 hours. Doubling of concentration did not increase the spike amplitude but rather increased the area beneath the curve. Porcine growth hormone treatment markedly increased plasma insulin concentration. Chung and co-workers (1985) also observed that pGH treated swine were hyperglycemic and concluded that the sensitivity of target tissues to insulin was decreased by pGH. Furthermore, plasma somatomedin C was not noticeably elevated by pGH nor did administration of GH result in pGH antibodies in any samples. In addition, chronic administration of pGH tended to increase plasma free fatty acids and significantly reduced blood urea nitrogen. Moreover, pituitary weight was not affected; however, both pituitary GH content and concentration were reduced more than 40%. Porcine GH related increase in plasma insulin was not associated with a change in serum glucose, and it appeared that part of the decrease in adipose tissue growth in pigs chronically treated with pGH was due to hormone effects on lipogenesis and insulin responsiveness of the adipocyte (Etherton et al., 1987). Peters (1986) administered bovine growth hormone to growing steers that were either limited or *ad libitum* fed and monitored lipid metabolism. He observed that free fatty acids

were higher in the restricted fed steers than the full-fed and treatment with growth hormone gave a further elevation in restricted fed steers but had no effect on full fed cattle. Insulin concentrations were higher in full fed cattle than in restricted fed cattle. Furthermore, treatment with growth hormone gave further elevation of insulin concentration in *ad libitum* fed but did not affect restricted fed. In addition, free fatty acid mobilization *in vivo* was higher in restricted fed cattle, with cattle receiving growth hormone having a greater free fatty acid increment in response to epinephrine. Insulin response to epinephrine tended to be higher in full fed steers and hormone treatment did not affect insulin release in response to epinephrine. Hormone treatment had no effect on *in vitro* rates of both basal and epinehrine-stimulated adipose lipolysis.

Many researchers have looked at the sensory attributes, qualitative characteristics and gross morphological structure of meat from GH treated animals with varying results. Researchers have found no significant effect of GH on sensory attributes of pork (Bechtel et al., 1988; Beerman et al., 1988a; Novakofski, 1988; Kanis et al., 1988; Prusa et al., 1989a). Bechtel and co-workers (1988) observed no effect of GH on juiciness and flavor intensity but they did see a small decrease in tenderness and a slight increase in off-flavors with GH treatment. Beerman et al. (1988) also reported no effect on the sensory attributes as well as no effect on Instron values and cook loss percentages. Solomon et al. (1989), on the other hand, observed that PST treatment resulted in an increase in shear-force of longissimus muscle, indicating

a reduction in meat tenderness when 100 μg $kg^{-1}$ $day^{-1}$ was administered between the weights of 30 and 60 kg with slaughter occuring when the animals reached 90 kg.

Few researchers have investigated the effect of PST on processing characteristics of muscle. Miller and co-workers (1989) observed the processing characteristics by marinating 100 g sections of *semimembranosus* muscle from PST treated pigs in sodium chloride, calcium chloride, EGTA and water, and vacuum tumbled for ten minutes. The muscle pieces were cooked, either immediately or after 3, 6, and 24 h storage to an internal temperature of 65°C. They found that samples from the PST treated animals required the greatest force to shear at 3 and 6 h; however, the PST samples were more tender than the control samples after aging for 24 h. Muscle pH values also were different at 3 and 6 h for the PST treated samples, but no differences occurred after 24 h storage. In addition, PST treated samples had higher purge losses and lower cooking losses than the control samples. Color, water-binding capacity and ultimate pH were unaffected by PST treatment (Miller et al., 1989). Beerman and co-workers (1988b); however, saw a dose-dependent increase in ultimate pH with PST treatment. Bechtel and co-workers (1988) observed alterations in muscle color and firmness, but the scores remained in the "acceptable" range. Furthermore, Ender et al. (1989a) observed an alteration in the fat from animals treated with PST. Fat quality was softer and contained increased unsaturated fatty acids, particularly C18:2 on a percentage basis (Ender et al., 1989a).

Changes in fiber types and area have been observed with PST treatment. Observations of the muscle fibers revealed that control pigs had fewer αR fibers and more αW fibers than animals treated with 100 $\mu g\ kg^{-1}\ d^{-1}$ of PST accounting for some of the changes in muscle color (Solomon et al., 1989). Furthermore, administration of PST increased muscle fiber area for all three fiber types. Solomon and co-workers (1989, 1990) reported that all PST pigs treated with 100 $\mu g\ kg^{-1}\ day^{-1}$ exhibited the "giant fiber syndrome" and a high proportion of PST treated pigs exhibited pale, soft, exudative muscle (62% of 14 animals).

The effect of GH on cultured muscle cells has been investigated. When added to cell culture at physiological levels porcine growth hormone had no effect on the proliferation of cultured muscle cells, however, slight stimulation of proliferation was observed at higher levels (> $10^{-4}$ M) (Kotts et al., 1987). Sera from pigs before and after injection with pGH stimulated proliferation to a greater extent than pGH alone. Proliferation was greater when sera from treated animals was used. Somatomedin-C levels were uniformly increased in sera in response to high doses (143 μg/kg/d) of PST to animals, suggesting that the observed proliferation of muscle cells in culture could be due to the treatment of animals with growth hormone (Kotts et al., 1987). The increased proliferation-promoting activity of sera obtained during and after porcine growth hormone injection suggests a possible role for somatomedins or other factors under the control of growth hormone in regulation of satellite cell proliferation in vivo (Kotts et al., 1987).

In general, exogenous growth hormone administration, increases growth rate and lowers carcass fat with out a major effect on the palatability of meat from treated animals. The main mode of action seems to be to alter metabolism to favor protein synthesis and limit lipid deposition. Differences in reported results are likely due to differences in doses given to animals and/or sources of growth hormone used, natural growth hormone extracted from the pituitary versus recombinant somatotropin (growth hormone).

## Porcine Stress Syndrome

Porcine stress syndrome (PSS) was first identified as a problem in the early 1960s. Selection pressure for lean, heavily muscled fast growing hogs resulted in an increase in unexplained death losses and lead to the identification of PSS. Symptoms of PSS include increased heart rate and respiration, flank and tail tremors, muscle rigidity, rapid rise in body temperature, metabolic acidosis, elevated serum metabolites and death (Topel et al., 1975; Cheah et al., 1984). This condition is similar to the human condition known as malignant hyperthermia (Jorgensen, 1985). A pale, soft, exudative (PSE) muscle condition is observed in those animals that did not die from stress.

Animals suffering from PSS have altered intracellular enzyme activities and hormone responses leading to changes in metabolism and ion homeostasis, especially calcium and magnesium. Stress susceptible pigs are unable to stabilize intracellular calcium levels within non-toxic ranges, and display increased magnesium deposition and decreased

urinary magnesium (Ludvigsen, 1985). High intracellular calcium stimulates actomyosin and therefore muscle contraction, while low magnesium results in the persistance of rigidity due to the inactivity of magnesium-stimulated myokinase (Ludvigsen, 1985). Administration of magnesium aspartate to stress susceptible animals decreased mortality during transportation (Ludvigsen, 1985). Calcium release from the sarcoplasmic reticulum is faster, and sustained longer in stress susceptible animals than in stress-resistant animals (Topel and Hallberg, 1985) leading to a rigidity different from rigor mortis. Rigor mortis is caused by a depletion in ATP and a fall in muscle pH, but the theorized cause of this rigidity is a sustained high level of myoplasmic calcium (Topel and Hallberg, 1985). Calcium accumulating ability of the sarcoplasmic reticulum decreases with time postmortem in both normal and PSE animals with loss of accumulating ability decreasing much more rapidly in PSE animals (Greaser et al., 1969). Cheah and colleagues (1984) observed that stress positive pigs showed significantly higher rates of calcium release from the mitochondria (219 nmol/min/mg protein vs 122 nmol/min/mg protein), with the maximum calcium release occurring within 45 min postmortem. They also showed that mitochondria isolated from stress positive animals contained a significantly higher endogenous calcium content (82 nmol/mg protein vs 45.4 nmol/mg protein).

There appears to be a close relationship postmortem between rate of pH decline in the muscle and loss of calcium accumulating ability in the subcellular fractions (Greaser et al., 1969). Also

decreasing pH further enhances uncoupling of sarcoplasmic ATPase (Topel and Hallberg, 1985). Cheah (1973) observed that mitochondrial activity declined during storage and the rate of decline was dependent on the tissue pH immediately postmortem and on its subsequent rate of decline. Cytochrome c lost upon postmortem aging was also dependent on the rate of pH decline. Topel and Hallberg (1985) observed that the respiration rate of mitochondria was not different between normal and PSE muscle, however, Eikelenboom and van den Bergh (1973) reported that mitochondria from PSE muscle had a reduced capacity for oxidative phosphorylation *in vivo*. Because of the reduced capacity, the aerobic pathways may be unable to oxidize the excess NADH formed by glycolysis and consequently glycolysis will become anaerobic in PSE pigs resulting in accumulation of large quantities of lactate (Eikelenboom and van den Bergh, 1973). Exposure to stress results in an increase in blood lactate and a reduction in high energy phosphate compounds in stress-susceptible animals (Eikelenboom and van den Bergh, 1973; Sair et al., 1972; Topel et al., 1975; Andersen et al., 1975; Essen-Gustavsson et al., 1988). It is assumed that the levels of energy-rich phosphates at the time of death are of primary importance in determining the rate of postmortem pH decline and the time course of rigor mortis, both of which are important variables between normal and PSE musculature in pigs (Eikelenbloom and van den Bergh, 1973). Besides the resulting effect on postmortem pH decline, lower levels of energy rich phosphates also

will decrease the efficiency of sarcoplasmic reticulum to bind calcium (Eikelenboom and van den Bergh, 1973; Essen-Gustavsson et al., 1988).

Potassium metabolism is also altered in stress susceptible pigs (Jorgensen, 1985). Low potassium supply results in highly significant delay in malignant hyperthermia and an almost complete attenuation of blood acidosis. Moreover, hyperkaliemia during malignant hyperthermia is a result of potassium loss from skeletal muscles and to a lesser extent from the liver (Jorgensen, 1985). A defect in muscle or one of its associated subcellular structures may form the basis for a possible cause of porcine stress syndrome (Topel and Hallberg, 1985).

Hormone concentrations are altered in response to stress in susceptible animals. Stress susceptible animals display 2-3 times higher ACTH levels than normal animals and metabolize cortisol five times faster leading to a lower plasma cortisol to ACTH ratio (Marple, 1977). During stress, epinephrine stimulates glycogen breakdown and blood glucose is elevated. Stress susceptible pigs have a significantly higher rate of glycolysis when exposed to stress than normal animals (Topel et al., 1975). Therefore, glycogen depletion is greater in muscle fibers from stress positive genotypes. Hormone levels may be an indication that the high susceptibility to stress is related to lowered adrenal cortex function or an abnormal clearance of hormones from the blood (Topel et al., 1975).

Dopamine, a neurotransmitter which inhibits excitatory neurons, has been implicated in brain responses to stress. Deficits of dopamine suggest that the overstimulation of the neuromuscular junctions during

stress may be the trigger which begins the cascade in muscle creating the final effects seen in PSS (Topel and Hallberg, 1985). Erlander and co-workers (1985) fed L-DOPA to stress-susceptible pigs to try and reduce the incidence of pale, soft and exudative meat. They monitored, before and after feeding of L-3,4-dihydroxyphenylalanine (L-DOPA), the levels of DOPA, dopamine, norepinephrine and ACTH in the hypothalmus and plasma. Stress pigs had greater norepinephrine levels in the hypothalamus and higher plasma ACTH levels than normal pigs. Furthermore, muscle pH was found to be lowered in stress pigs accompanied by a higher muscle reflectance and a 75-fold increase in plasma DOPA. L-DOPA feeding eliminated strain differences in brain catecholamines and their metabolites. This indicated to the researchers that chronic L-DOPA feeding could equalize the stress response of brain catecholamines in stress pigs and normal pigs. Pale, soft and exudative meat, however, was still present in L-DOPA supplemented animals. This observation suggests that even though L-DOPA supplementation eliminated strain differences with regard to brain catecholamines, feeding of L-DOPA did not alter skeletal muscle function to the degree of diminishing the development of pale, soft, exudative pork.

PSE muscle displays altered activity of a variety of enzymes, protein solubility and structure. Genetic type affects the solubility of sarcoplasmic proteins and the activities of creatine phosphokinase and phosphofructokinase (Talmant and Monin, 1983). Although no differences in ATPase activity in myofibrils, mitochondria, heavy sarplasmic reticulum and light sacoplasmic reticulum were found

between normal and PSE muscle immediately postmortem, ATPase activity postmortem of PSE myofibril dropped significantly within 30 minutes after death. By 24 hrs, it dropped to a level that was less than half the initial activity (Greaser et al., 1969).

Schwagele and Honikel (1988) found no change in glycolytic enzyme concentrations but the activity of phosphorylase, adenlyate kinase, and pyruvate kinase increased. They observed that the activity of phosphorylase a and b from muscles with a 45-minute pH between 5.5 and 6.1 was five times higher than in the range above 6.1. Phosphofructokinase showed no activity difference in the pH range observed, but both pyruvate kinase and adenylate kinase are more active in pH range below 6.1. Because of these results the researchers concluded that the development of the PSE-syndrome, among other parameters, depends on the representation of isoenzymes involved directly in the turnover of energy rich compounds during glycogenolysis. Severini and co-workers (1984) observed that the PSE condition in muscles was characterized by the rapid fall in pH, rapid breakdown of glycogen by the phosphorylase activity, rapid production of a large quantity of lactic acid at high temperature, and low water-holding capacity just one hour after death. The pH seems to be related to the glycogen content in the muscle just before slaughter as well as the rate of glycolysis. Muscles with pH less than or equal to 5.59 one hour after slaughter had temperatures greater than 41.5°C, a large amount of lactic acid (93 $\mu$M/g) and a pale, soft appearance under these conditions (Severini et al., 1984). Furthermore, the glycogen and

phosphorylase activity were almost absent from PSE muslce one hour postmortem (Severini et al., 1984).

PSE muscle also is characterized by different fiber types which may contribute to the PSE syndrome. The different fiber types in the muscle undergo glycolysis at different rates. White fibers have a higher rate of glycolysis than red and intermediate fibers. Sair and colleagues (1972) observed that stress susceptible animals have a larger number of intermediate fibers, an increase in the area of dark and white fibers, and a positive correlation between 45 minute pH values and the amount of white fibers. Essen-Gustavsson and co-workers (1988) observed that fiber types were not different between stress genotypes, but mean fiber area was larger and capillary density was lower in stress positive genotypes. Ruusunen and Puolanne (1988) observed that during stress only white fibers metabolize glycogen. Furthermore, Essen-Gustavsson and colleagues (1988) observed no difference in the percentage of glycogen depletion of type I (red) fibers, but almost twice as many type IIA (intermediate) and IIB (white) fibers were glycogen depleted in stress susceptible pigs. Glycogenolysis was markedly increased in type IIB fibers with a positive correlation seen between the percentage of depleted IIB fibers and both muscle lactate and ammonia concentration, however, negative correlations to both ATP and CP concentrations were observed (Essen-Gustavsson et al., 1988). They suggested that the increase in ammonia indicated an increase in nucleotide turnover (Essen-Gustavsson et al., 1988).

The observed pale, watery condition of PSE muscle has been widely studied and certain events postmortem are known to contribute to the visual changes. McLoughlin and Goldspink (1963b) observed that loss of color was associated with a rapid fall in pH after death and suggested that the color of muscle myoglobin was masked by heavy protein precipitation. They also reported that carcasses appear normal up to 45 min postmortem, with adverse changes in texture, color and water-holding capacity properties becoming apparent during subsequent cooling of the carcass (McLoughlin and Goldspink, 1963a). Exudate or drip increases as the time interval, temperature and rate of pH decline increases postmortem and is associated with a decrease in protein concentration in the drip and a decrease in ATPase activity indicating denaturation of myofibrillar proteins (Penny, 1977; Warriss, 1982). However, muscle from stress pigs held at 10°C during rigor showed normal drip loss. In addition, an increase in extracellular space was associated with an increase in drip loss, also extracellular space increased with time postmortem (Penny, 1977). Myofibrils, like whole muscle from PSE animals have characteristic changes. Myofibrils, washed with 0.04 M phosphate buffer, from meat with low 45 minute pH values displayed a higher protein content but a lower water retention per gram of protein (Bendall and Wismer-Pedersen, 1962) than muscle with a 45 min pH higher than 6.0. Furthermore, extraction of protein, using a phosphate buffer with an ionic strength of 0.55, was almost complete from myofibrils isolated from normal muscle (88.5%),

while only 11% protein was extracted from myofibrils isolated from PSE muscle (Bendall and Wismer-Pedersen, 1962).

Sayre and Briskey (1963) observed a significant correlation between sarcoplasmic protein solubility and pH at onset and completion of rigor. Muscle temperature at death was also closely related to sarcoplasmic protein solubility. Furthermore, they observed a significant correlation between myofibrillar protein solubility and initial temperature, and a significant correlation between myofibrillar protein solubility and muscle temperature at onset of rigor (Sayre and Briskey, 1963). Muscle that ultimately became exudative began to lose sarcoplasmic protein solubility immediately after death, but myofibrillar protein solubility was not affected as rapidly (Sayre and Briskey, 1963). Bendall and Wismer-Pedersen (1962) observed that "watery" fibrils (PSE) retain less water per gram of protein than those of normal pork at all pH values between 4.5 and 7.5. Although they observed a zone of minimal swelling that was the same in both normal and PSE muscle, the zone is wider for "watery" fibrils and extends to a lower pH. This is the opposite of what happens to proteins aggregated by heat. Bendall and Wismer-Pedersen (1962) concluded that the immediate cause of wateriness is the combined effect of high temperature and low pH.

Aside from their decreased extractability and water retention, watery fibrils are distinguishable from normal fibrils mainly in their higher protein content. Bendall and Wismer-Pedersen (1962) suggested that this extra protein is from the aggregation of

sarcoplasmic proteins with myofibrillar proteins and not denaturation or coagulation since titration curves of the proteins from both normal and PSE were similar. In addition, Scopes and Lawrie (1963) observed that several protein components of the sarcoplasmic protein complex are unstable and either denature, or are isoelectrically precipitated by the postmortem pH fall in muscle. The effects of low pH and high temperature postmortem caused precipitation of soluble sarcoplasmic proteins since the solubility decreased moderately to extensively during the first 24 hr postmortem, regardless of physiological conditions in the muscle at onset of rigor (McLoughlin and Goldspink, 1963b; Sayre and Briskey, 1963). Muscle with a rapid pH decline had decreased sarcoplasmic protein solubility at 24 hr postmortem to 55% of original value (Sayre and Briskey, 1963). In support of decreased sarcoplasmic protein solubility, water extracts of normal muscle were more turbid than water extracts of PSE muscle indicating lowered solubility of sarcoplasmic proteins (Hart, 1962). McLoughlin and Goldspink (1963b) observed that the proteins in exudate are identical to sarcoplasmic proteins extracted from muscle on electrophoretograms. Myofibrillar proteins showed no loss in solubility under conditions of slow or medium pH decline but under conditions of high temperature and medium or rapid pH decline, loss of myofibrillar protein solubility was severe (Sayre and Briskey, 1963). Less than 50% of the fibrillar protein was extractable at onset of rigor and only 25% was extractable after 24 hr.

Stabursvik and co-workers (1984) used differential scanning calorimetry (DSC) to determine the degree of denaturation of myofibrillar proteins, actin and myosin, in PSE muscle as compared to normal muscle. DSC thermograms of myofibrillar tissues from normal pork display three major peaks with temperature maxima ($T_{max}$) at 58, 66 and 78°C. They observed that PSE reduced the $T_{max}$ at 58°C by half and the $T_{max}$ at 66°C by approximately 15% but the $T_{max}$ at 78°C was only reduced about 5%. The $T_{max}$ at 58° and 66°C are related to myosin while the $T_{max}$ at 78°C is related to actin. Subfragment 1 (S-1) contributes to the myosin peak at 58°C. Also, the ATPase activity is associated with S-1 fraction. The decrease in ATPase activity and the decrease in $T_{max}$ 58°C indicated to these researchers that the S-1 fragment of myosin was denatured in PSE muscle (Stabursvik et al., 1984). They concluded that the PSE condition is primarily characterized by extensive denaturation of part of the myosin molecule or, an alternative explanation involves the total denaturation of approximately 50% of the myosin molecules while the remaining molecules remain intact.

Kang and co-workers (1978) purified myofibrils from normal and PSE animals to observe changes in the Z-line and other degradation changes. Myofibrils from PSE muscle showed little fragmentation postmortem and they were more resistant to fragmentation when purified calpain was added *in vitro*. Calpain, added to myofibrils, digest more normal myofibrils than PSE myofibrils, based on the release of soluble material and fragmentation of myofibrils.

Deoxycholine (DOC) is reported to selectively extract Z-line components (Kang et al., 1978). DOC extracts less material from PSE muscle than from normal muscle. It appeared that troponin T from PSE myofibrils was more resistant to degradation than that from normal myofibrils (Kang et al., 1978). In addition, less α-actinin was extracted with DOC from PSE myofibrils (Kang et al., 1978). PSE muscle also showed slower rate of Z-line removal with no apparent difference in the myofibrillar constituents (Kang et al., 1978). Alpha-actinin and troponin-T disappeared from normal myofibrils at a much higher rate than from PSE myofibrils (Kang et al., 1978). Moreover, DOC extractability of α-actinin was correlated with degradation of Z-line structure and with differences in fragmentation (Kang et al., 1978). Decreased Z-line degradation could be due to masking of the structure or to alteration of the structure (Kang et al., 1978). From these observations, Kang and colleagues concluded that 1) Z-line structure itself or Z-I junction in PSE muscle are more resistant to calpain than normal, or 2) calpain from PSE muscle has a lower activity or is more labile than that of normal muscle.

Connective tissue changes have been suggested because of the looser muscle structure (Hart, 1962; McClain et al., 1969). McClain and co-workers (1969) studied collagen from PSE animals and observed that there was more salt soluble collagen in PSE tissues but no difference in amount of acid soluble collagen. This was thought to be due to an increase in tropocollagen or possibly more immature collagen. Furthermore, there was a decrease in intramolecular cross-

links and an increase in heat labile collagen in PSE tissue. However, no alteration in amino acid composition was seen in collagen from normal and PSE muscle. Hart (1962) observed, however, no differences between collagen of PSE and normal muscle.

Sensory, proximate composition and processing characteristics are affected by the PSE condition. Merkel (1971) found no difference in proximate composition of loins between normal and PSE. Sayre and co-workers (1964) also reported no difference in the proximate composition between normal and PSE muscles. In addition, Merkel (1971) found that normal roasts took significantly longer to cook than PSE loins and PSE roasts were scored significantly lower for juiciness, but overall acceptability between the two kinds of roasts was not significantly different. Furthermore, Warner-Bratzler shear values were significantly lower for PSE loins. Emulsifying capacity was significantly lower for PSE muscle, and PSE hams showed significantly lower processing yields than normal hams (Merkel, 1971). Moreover, PSE hams had significantly lower acceptability scores, had a "mushy" texture, tasted saltier than normal hams and displayed a paler color (Merkel, 1971). Wismer-Pedersen (1960) observed that meat from PSE animals bound less water, more nitrogen compounds diffused out of the meat and the salt content was consistently higher in processed hams from PSE muscle. In addition, Wismer-Pedersen (1960) observed that color, taste and texture were inferior in hams from meat with watery structure. He also showed that the pale color was not due to pigmentation concentration differences. Sayre and co-workers (1964)

reported that the pH at onset and completion of rigor was highly correlated with the evaporative cooking loss, cooking rate and tenderness of muscle. Furthermore, the solubility of sarcoplasmic and myofibrillar proteins was highly associated with cooking evaporation (Sayre et al., 1964). Topel and colleagues (1976) reported that consumers preferred normal chops to dark or pale chops based on visual appraisal. Furthermore, the color of pale chops was less stable under retail storage conditions than normal chops (Topel et al., 1976). Moreover, Topel and co-workers (1976) reported that broiled pork chops from normal carcasses were preferred to chops from PSE carcasses by consumer and trained panelists.

Muscle quality is affected by many factors. Barton-Gade (1988) observed the effect of breed on meat quality and processing characteristics of the muscle, and reported that a lower ultimate pH in Hampshire pigs lead to decreased color and water-holding capacity. Overall, Landrace had the highest incidence of both pale, soft, exudative (PSE) and dry, firm and dark (DFD)-meat, followed by Large White. The Duroc breed had the highest ultimate pH and few had PSE-/DFD meat. Barton-Gade (1988) also observed that PSE-meat with an intramuscular fat content below 2% generally had unacceptable eating quality whereas non-PSE meat with sufficient marbling (2% and above) generally had excellent eating quality. Overall, Landrace had the best characteristics for processing, even though the PSE-frequency is higher than in other breeds, with higher processing yields and lower fat levels in the finished product, allowing the declaration of maximum 2% fat in

hams along with a darker color due to increased pigment content. The Duroc breed has major disadvantages for processing because of a much higher degree of marbling and a lowered protein and pigment content (Barton-Gade 1988). Besides breed differences, Eikelenboom (1985) suggested four things to do to improve meat quality in pigs: 1) Halothane testing of breeding stock to remove porcine stress syndrome animals from the herd, 2) genetic selection for meat quality, 3) objective measurements, and 4) improved pre-slaughter handling, with at least a 2-4 hour rest. Webb and co-workers (1985) demonstrated that selection based on halothane testing can reduce the incidence of halothane positive animals in the herd. They also suggest that halothane locus is affected by both genetic and environmental factors, but is predominantly a complete recessive gene. Eikelenboom and Nanni Costa (1988) found that halothane positive pigs showed a more rapid postmortem pH decline, and onset of *rigor mortis* as well as higher muscle temperature. Furthermore, after slaughter the carcasses from halothane positive animals have the fastest pH decline, lightest color and poorest water-holding capacity (Jensen and Barton-Gade, 1985).

Many methods have been developed to help identify PSE carcasses rapidly after slaughter. Eikelenboom and Nanni Costa (1988) used a fibre optic probe to identify PSE carcasses. The fibre optic probe measurements taken at 45 minutes postmortem were largely affected by halothane phenotype, and a high correlation was observed with other measurements such as drip loss and 45-minute pH.

Swatland (1986) also used a fiber optic reflectance probe to determine quality of pork carcasses. He observed that reflectance values increased during the course of postmortem metabolism. Muscle from PSE carcasses had higher reflectance values at all wavelengths tested relative to normal loins, with measurements made from 600-700 nm related to CIE%Y (reflectance), pH and subjective color. Increased or decreased pigment content can significantly decrease or increase reflectance values respectively (Lundstrom et al., 1988). Instruments using internal reflectance values show only low or non-significant relationships with pigment, and the solubility of the total proteins explains the highest proportion of variation for all instruments used to identify PSE carcasses (Lundstrom et al., 1988). Surface reflectance is based on total pigment content (Vada-Kovacs et al., 1982) and this makes it unsuitable for evaluation of meat quality. Furthermore, percent transmission as a measure of protein solubility is influenced somewhat by 45 minute pH and mostly by the ultimate pH (Vada-Kovacs et al., 1982). Swatland (1985) also tried capacitance and observed that the values declined continuously postmortem. Jones an co-workers (1984) observed that capacitance was more related to final meat color than initial pH and that one hour reflectance had the lowest relationship with 24 hour paleness. Ockerman and Cahill (1968) extracted proteins and measured the transmittance of the solution. They observed that all samples with high transmission values (> 10) were from carcasses rated by a panel as slightly pale to slightly pale, soft with less marbling. This method could identify poor quality

carcasses, but is not practical in a slaughterhouse setting. Honikel and Fischer (1977,1980) determined a R value for carcasses. The R value is a ratio of absorbance at 250 to 260 of a perchloric extraction of muscle and is influenced by the ATP present in the sample. This value, in conjunction with 45-min pH can clearly classify PSE, DFD and normal muscle within 1 hr postmortem; however, the procedure is tedious and too slow for slaughter line use. The ATP content as a predictor of PSE is related to color and pH, but does not contribute anymore to the prediction because the complexity of the procedure makes it impractical to use in slaughter houses (Yang et al., 1984).

## Water-Holding Capacity

Water constitutes approximately 75% of the weight of lean meats, and the ability of the muscle to bind water is of scientific and economic importance. It is of practical and scientific importance because it is closely related to palatability, tenderness, color and several processing characteristics (Hamm, 1960). Water-holding capacity is of economic importance because meat and meat products are sold by weight and water lost from products results in economic loss to the processor.

Hamm (1960) concluded that water in muscle can be classified as bound, immobilized and free. Bound water constitutes approximately 4% of the water in meat and is bound very firmly to certain polar, hydrophilic groups on the muscle proteins and is diminished when these polar groups, undissociated carboxyl groups and amido groups are blocked. This water is very tightly held and can be removed only under severe drying conditions. The majority of water is held loosely

by hydrogen bonding between water molecules and by capillary action. That water held by capillary action can easily be removed by drying, drip loss or other physical force. Water-holding capacity is affected by changes in protein charges (Hamm, 1960). The amount of "free" water immobilized within tissue is strongly influenced by the spatial structure of the muscle tissue (Hamm, 1960). Minimal water-holding capacity is observed as the pH approaches the isoelectric point of actomyosin so there is a minimum charge on the protein and no interaction with water takes place. Small changes in meat pH may cause relatively great changes of water-holding capacity (Hamm, 1960). The decrease in protein solubility with decreasing pH in the pre-rigor phase parallels the pH dependence of protein solubility in the rigor muscle homogenates (Honikel et al., 1981a). With the falling pH cooking loss increases. Furthermore, the onset of rigor causes a remarkable decrease in WHC of salted muscle, but has no effect on unsalted meat (Honikel et al., 1981a). Honikel and co-workers (1981b) observed that longitudinal changes of muscle that occur during contraction have much less influence on WHC than transversal alterations as caused by attraction between oppositely charged groups of adjacent protein molecules and particularly by cross-linking between myofilaments during development of rigor. High water retention after cooking was accompanied by high expressible juice values. Moreover, chronological age of animals has no effect on WHC or residual bound water (Bouton et al., 1972). Stretched muscle displays higher WHC than their cold shortened counterparts (Bouton et al.,

1972). Severini et al., (1984) observed that one hour postmortem pH below 6.0 was related to a lower average water-holding capacity, and an increase in pale color was also related to a decrease in water-holding capacity. Warriss and Brown (1987) found a linear relationship between pH at 45 minutes postmortem, but there was a break at 6.1, that showed an increased drip with a decreased 45 minute pH. Penny (1969) also observed that water-holding capacity, extractability and calcium ATPase activity were unaffected by the 90 minute pH values between 5.9 and 6.9, but when the pH fell below 5.9, the results for all three parameters decreased. He concluded that the water-holding capacity of myofibrils and hence intact meat is very largely determined by the extent of denaturation of the myofibrillar proteins. Protein extractability accounts for approximately 82.4% of the total variation in water-holding capacity (Penny, 1969). Paterson and co-workers (1988) observed that increased water-holding capacity in salt and phosphate treated muscle was associated with an increased solubility of titin and myosin heavy chains. They also saw that increased water-holding capacity was associated with an increased swelling of myofibrils. They concluded that the regulation of water-holding capacity was presumably due in part to removal of structural restraints of titin containing structures. Offer and Trinick (1983) also observed that water-holding capacity was increased by the removal of transverse constraints of the intact myofibril.

Kauffman et al. (1986) compared various methods of measuring water-holding capacity and found that none of the cooking loss

methods tested could distinguish between PSE and normal pork muscle. Furthermore, transmission tests, percent fluids and the imbition test (measure of absorption of fluids on pH paper within 3 min. of exposure to freshly cut meat surface) could not distinguish normal muscle from DFD muscle. High repeatability was reported for the three drip loss methods, cook loss from packaged slices, three laboratory category methods, two filter paper absorption methods and permitivity test capacitance ratio (electrical and dielecric constants at two energy frequencies). The most variable procedure for determining water-holding capacity were the filter press methods.

## Sensory Evaluation

Sensory evaluation is a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials which are perceived by the senses of sight, smell, taste, touch and hearing (Reaume, 1975). The sensory perception of a sample is a combination of flavor, appearance and kinesthetics. Flavor is a combination of the four basic tastes, sweet, sour, salty and bitter (Meilgaard et al., 1987a) with smell (Kramer, 1973). Appearance is the size, shape and color (Meilgaard et al., 1987a) of the sample, while, kinesthetics of a sample is its texture, viscosity and mouth feel. The role of sensory panelists as measuring devices is analogous to the use of any scientific instrument. Therefore, panelists should be trained and be actively involved in the development of scales used to measure the panelists response (Meilgaard et al., 1987a)

There are three fundamental types of sensory panels with three very different functions (Abbott, 1973). Preference/acceptance panels evaluate the opinions or likes and dislikes of the individual and should be conducted using consumers. Preference tests determine which of 2 or 3 samples most people like best. Acceptance tests determine which product is most likely to be accepted by the consuming public. Difference tests determine if there is a detectable difference among samples. They do not indicate the magnitude or type of difference, only if the samples are identical. Descriptive tests indicate the kinds or size of differences among samples. The results of a majority of sensory panels reported in the literature are intended to be of a descriptive nature because the researcher is interested in the effects of the studied variables on the food (Meilgaard et al., 1987a).

Sensory evaluation is important in meat research. The most common sensory tests used in meat science research include hedonic rating scale, a preference/acceptance test, and scoring, a descriptive test. Hedonic rating is where the panelist indicates their degree of like or dislike for a specific attribute or the product in general (Meilgaard, et al., 1987b). In scoring or rating the relative intensity or amount of a property is indicated by a mark on a rating scale or by a numeric score (Abbott, 1973; Meilgaard et al., 1987b). Some researchers prefer scales without numbers because they feel that these scales eliminate possible bias for specific numbers and gives a continuous scale (Stone et al., 1974).

Control of environmental factors is very important in sensory work with food. The room should be quiet, free from interruptions and distractions (Meilgaard et al., 1987a). Conditions in the laboratory should be uniform for the sake of comparable performance from day to day and sample to sample. Among the environmental conditions to be considered are air conditioning/heating, lighting and seating comfort (Meilgaard et al., 1987a).

Sample preparation and service should be standardized. The normal procedure is to test the food under conditions approximately the same as those of normal consumption (Meilgaard et al., 1987a). The general philosophy is that the samples presented should be exactly the same with respect to all of the factors under experimental control (Meilgaard et al., 1987a). Uniformity of samples presented to panelists is important. Among factors to be considered are visual appearance, sample size, temperature, coding, order, instructions to panelists, and palate rinsing.

## Cookery

The method of cookery largely determines the effect of heating on muscle, especially whether the toughening effect of heat on myofibrillar proteins and on elastin will be offset by its tenderizing effect on collagen (Laakkonen, 1973). Shrinkage during heating is an important characteristic of muscle proteins (Laakkonen, 1973; Davey and Winger, 1980). The meat fiber retains its striated appearance upon cooking, even for prolonged periods at high temperatures (Davey and Winger, 1980); however, some changes in the ultrastructure occur.

Fine detail is lost from the A-band which shrinks in length from 1.5 to 1.1 μm. Furthermore, thin filaments lose much of the recognizable structure and appear to clump together through side to side aggregation. Gap filaments may still remain intact but are unidentifiable in the debris. In addition, Z-discs become more granular and diffuse, and the sarcoplasmic reticulum largely disappears, although empty organelles persist in the spaces between myofibrils (Laakkonen, 1973; Davey and Winger, 1980). Intermolecular cross-links of collagen are thermal labile. As the animal gets older fewer of the cross-links are labile and accounts for the decrease in solubility and increase in the shrinkage temperature with age (Laakkonen, 1973). Elastin is a rubberlike protein that occurs in connective tissue in relatively small amounts (Laakkonen, 1973). It, like myofibrillar proteins, shrinks and hardens upon heating (Laakkonen, 1973).

Hamm (1960) pointed out that meat will be more juicy, (less juice that is released during cooking and the more tightly bound to the coagulated tissue) and that less water is released when muscle is cooked at a lower temperature and to a lower end-point temperature. Shrinkage and moisture loss during cooking seem to toughen the meat. Internal temperatures of about 70°C and above seem to have a toughening effect, since they cause severe shrinkage of meat proteins followed by squeezing out of meat juices (Laakkonen, 1973). At about 60°C the shrinkage seems to be at a minimum.

Parrish and co-workers (1973) reported that internal temperature was an important effector of palatability and cooking loss

and as the internal temperature increased, the palatability characteristics of flavor, tenderness, juiciness and overall acceptability decreased, and percentage cooking loss increased, in a linear manner. These researchers also found that the most desirable rib steak was obtained when broiled to 60°C internal and that greater internal temperature only reduced palatability attributes of the rib steaks (Parrish et al., 1973). Furthermore, Heymann and co-workers (1990) reported that the optimum end-point temperature for fresh pork roasts should be at least 71.1°C and should not exceed 76.7°C. They observed that sensory scores for tenderness and juiciness decreased with increased end-point temperature but pork flavor scores increased. Fjelkner-Modig (1986) also saw improvement in tenderness and juiciness scores when pork was cooked to lower end-point temperatures.

# SECTION I: EFFECT OF PORCINE SOMATOTROPIN, STRESS SUSCEPTIBILITY AND FINAL END POINT OF COOKERY ON THE SENSORY, PHYSICAL AND CHEMICAL PROPERTIES OF PORK LOIN CHOPS

TITLE: Effect of porcine somatotropin, stress susceptibility and final end point of cookery on the sensory, physical and chemical properties of pork loin chops

AUTHORS J. A. Boles, F. C. Parrish, Jr., C. L. Skaggs, and L. L. Christian

AFFILIATION: Departments of Animal Science and Food Technology, Iowa State University, Ames, IA 50011

RUNNING TITLE: Effect of PST on pork chops

## ABSTRACT

Forty-eight pigs of three known stress genotypes were injected daily with recombinant porcine somatotropin (PST) (4 mg/day) or placebo. The effect of PST and stress classification on the sensory, physical and chemical characteristics of pork chops were observed. Two end-point temperatures (71° and 77°C) were used for sensory analysis.

PST treatment significantly decreased panel scores for tenderness, juiciness and flavor, while stress susceptibility decreased panel scores for tenderness only. PST treatment reduced intramuscular fat and increased moisture in the *longissimus* muscle, but PSE had no effect on proximate composition. PST treatment and stress susceptibility decreased and increased Hunter L values of chops, respectively. Furthermore, greater end-point temperature reduced sensory scores for tenderness and juiciness.

## INTRODUCTION

Recently there has been an increased consumer demand for lean meat, and a goal of the pork industry is to discover ways of producing leaner pork. Recent research has focused on the use of recombinant growth hormone (porcine somatotropin, PST) to increase growth rate and feed efficiency, and reduce carcass fat. Chung et al. (1985), for example, have observed a decrease in fat and an increase in protein of pork carcasses. Few researchers have investigated the sensory attributes of pork chops (*longissimus* muscle) from pigs administered growth hormone during growth and development. A major question to be answered concerns the effect of PST on the palatability and processing properties of meat and meat products. Also, the effect of PST on stress susceptible swine needs to be explored. Some researchers have reported an increase in pale, soft and exudative (PSE) pork with the administration of PST at a level of 100 $\mu g\ kg^{-1}\ day^{-1}$ and slaughtered at lighter weights (90 kg) (Solomon et al., 1989; Solomon et al., 1990). Unfortunately, PSE muscle is usually associated with stress susceptible animals (Topel et al., 1975; Cheah et al., 1984). Therefore, the effect of PST on stress susceptible animals needs exploring. Another area requiring investigation is the effect of end-point temperature on the palatability of chops from PST treated and PSE animals. The objective of this study was two-fold. One was to determine the effect of PST and stress genotype (PSE) on the

palatability, proximate composition, and lean color of pork loin chops. The other was to determine the effects of final end-point temperature of cookery on the palatability of pork loin chops.

## MATERIALS AND METHODS

Forty-eight crossbred pigs (24 barrows, 24 gilts) were allocated to six treatment groups consisting of eight head (4 barrows, 4 gilts) based on stress classification, and Pitman-Moore (PM) porcine somatotropin (PST) or placebo administration. The research trial consisted of two replicates based on availability of animals of the different stress classification genotypes within each litter. When possible littermates were assigned to each treatment combination. Stress classification was determined by halothane screening (Christian, 1974), creatine phosphokinase (CPK) (Allen and Patterson, 1971), and blood typing (Rasmusen and Christian, 1976). Groups were designated as follows: stress-positive placebo (SP); stress-positive PST (SPPST); stress-carrier placebo (SC); stress-carrier PST (SCPST); stress-negative placebo (SN); stress-negative PST (SNPST). Pigs were fed *ad libitum* a corn-soybean diet and housed in a confinement building, with two pigs allotted per 1.22 x 3.35 m pen. Pen assignment was done before pigs weighed 31.8 kg. Pens were started on test when total pen weight reached 109 kg., and at this weight injection of recombinant PST (4 mg of PST/day) or placebo (diluent of PST treatment) was initiated. Each pig was injected in the neck once daily until taken off test. PST treatment was terminated at weekly intervals as individual pigs reached 109 kg., but animals continued to be fed for 6 additional days to allow for required withdrawl time. Pigs, after an overnight fast, were slaughtered at Iowa State University Meat Laboratory. *Longissismus dorsi* (LD) samples were collected 45-min post-slaughter

for determination of muscle pH (Warriss, 1982) to confirm stress classification. After a 24-hr postmortem chill (4°C) primal loins and *semimembranosus* (SM) muscle samples were obtained from one side, vacuum-packaged and frozen at -29°C.

Sensory Evaluation of Loin Chops

The sensory panel consisted of thirteen trained panelists. Panelists were trained to evaluate the sensory attributes of broiled chops for initial juiciness, sustained juiciness, initial tenderness, sustained tenderness and cooked pork flavor. Initial juiciness was defined as the sensation of amount of increased free fluids in the oral cavity during the first bite. Sustained juiciness was the maintenance of free fluids in the oral cavity. Initial tenderness was the force required by the molar teeth to bite through the meat fibers on the first bite. Sustained tenderness was the resistance to breakdown on further mastication. Pork flavor was the intensity of the flavor of cooked (broiled) pork perceived. Training consisted of presenting the panelists with samples of known juiciness, tenderness and flavor. Panelists scored each sample for initial tenderness, sustained tenderness, initial juiciness, sustained juiciness and flavor by using a 150-mm unstructured line scale. The scale was anchored 10 mm from the left end with a vertical mark and a descriptive term that represented a low score for juiciness, tenderness or cooked pork flavor intensity. A vertical mark and descriptive phrase 10 mm from the right end of the scale represented a high score for sensory attributes. Coded samples were presented during training to monitor panel performance.

Triangle tests were used also to assess the ability of panelists to determine differences in juiciness, tenderness and flavor.

Pork chops (1.91 cm) were cut from the frozen primal loin and thawed for 24 hrs at 2°C, and cooked by placing the chops on broiler pans four inches from the heat source of an electric broiler (204°C), and were turned half way (35° or 38°C) through cooking. The end-point temperatures of doneness used were 71° and 77°C. To achieve these end-point temperatures chops were removed from the broiler when the internal temperature had reached 68° and 74°C, respectively, as determined by thermometers inserted into the center of each chop. Cooked chops were sectioned into 1.27-cm cubes immediately after cooking and these cubes were placed in preheated, individually coded glass jars (44 mL). Panelists were seated in individual booths illuminated with red lights and had room temperature water for clearing the palate between samples. The scores of each individual panelist were used for statistical analysis.

Four chops from each treatment combination were cooked for cook loss determination. Two chops were broiled to 71°C and the other two were broiled to 77°C. Raw and cooked weights were recorded and used for cook loss determination. To determine the percentage cook loss the difference between raw and cooked weight was divided by the raw weight and multiplied by 100.

$$\frac{\text{raw weight - cooked weight}}{\text{raw weight}} \times 100 = \text{cook yield}$$

## Proximate Analysis

Chops (1.91 cm) from approximately the 10-12 rib section were minced in a Cuisinart food processor for 1 min and the mince was transferred to Whirl Pak™ bags and frozen. The homogenated muscle samples were thawed and subsequently used for proximate analysis. Moisture, fat and protein content of the loin chops were obtained by following AOAC (AOAC, 1984) procedures used in the ISU Meat Laboratory. The mean of three measurements was used for statistical analysis.

## Color Measurements

Color measurements for uncooked loin chops were made by using a Hunter Labscan equipped with a fluorescent light source and a 1.27 cm aperture opening. Measurements (L, a, b) were made on samples overwrapped with polyvinylchloride wrap. The mean of three measurements was used for statistical analysis.

## Instron Measurement

In an attempt to quantify the softness of pale, soft, exudative chops, five-cm squares were cut from the center of 1.91 cm thick chops and used for compression analysis on an Instron Universal Testing Machine. Conditions used with the Instron were: a 50 kg load cell, a crosshead speed of 200 mm/sec, a chart speed of 500 mm/sec and full scale was set at 5 kg. Each sample was compressed to 37.5% of its original thickness. The maximum force generated (peak height) was

used as an estimate of the firmness of the sample. Three chops from each animal were tested and the mean of the three measures was used for statistical analysis.

## Statistics

The design of this study was a 2x3 factorial design. But, to utilize the two end-point temperatures, a split plot analysis was also used for sensory and cook loss of loins. The two factors in the experimental design were two PST treatments and three stress genotype classifications. Significance of the main effects of PSE classification and PST treatment were tested against the individual animal variation within the PSE/PST treatment combinations for the sensory data. Temperature effects were tested against the individual animal variation within the PSE/PST/Temperature treatment combinations. Analysis of all data was performed by using the Statistical Analysis System (SAS Institute Inc., Cary N. C.). The general linear model procedure was utilized, and guidance for model statements and tests of significance was obtained from SAS Institute Inc. (1985a, b).

## RESULTS AND DISCUSSION

Chops from PST treated pigs received significantly ($P<.05$) lower scores for initial tenderness, initial juiciness, sustained juiciness and flavor than placebo (control) chops (Table 1). The decreased initial tenderness suggests an increase in myofibrillar toughness in contrast to sustained tenderness which represents connective tissue toughness (Szczesniak, 1986). In a similar study to the one reported herein pork chops from PST treated animals (4 mg/day) showed no difference from controls in juiciness, tenderness or flavor (Prusa et al., 1989). Novakofski and co-workers (1988) and Kanis et al. (1988), also found no changes in sensory characteristics of pork chops between PST treated and control animals. In this study, there was neither an interaction between PST treatment and stress genotype, nor was there an interaction between PST and end-point temperature. The positive stress genotype significantly ($P<.05$) decreased initial tenderness and sustained tenderness, but had no effect on initial juiciness, sustained juiciness or flavor (Table 2). This does not agree with results reported by Andersen and colleagues (1975) in which they found no differences between chops from normal and PSE animals for tenderness, juiciness or flavor. The greater end-point temperature (77°C) significantly decreased all sensory attributes except flavor (Table 3). That is, chops broiled to 71°C internal temperature of doneness were more tender and juicy than those broiled to 77°C. Heymann and co-workers (1990), however, saw an increase in pork flavor when roasts were cooked to higher end-point temperature (82.2°C). They also observed a decrease

Table 1: Mean sensory values[z] of loin chops from placebo (control) and porcine somatotropin-supplemented pigs

| | SENSORY ATTRIBUTE | | | | |
|---|---|---|---|---|---|
| PST TREATMENT | INITIAL TENDERNESS | SUSTAINED TENDERNESS | INITIAL JUICINESS | SUSTAINED JUICINESS | FLAVOR |
| Placebo | 70.3±1.3[a] | 72.2±1.4[a] | 84.8±1.3[a] | 85.1±1.2[a] | 77.6±1.2[a] |
| PST | 62.7±1.3[b] | 65.3±1.3[a] | 72.4±1.4[b] | 73.2±1.4[b] | 72.0±1.2[b] |

[z]0 = least intensity of juiciness, tenderness, or pork flavor; 150 = greatest intensity of juiciness, tenderness, or pork flavor.

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

Table 2: Mean sensory values[z] of loin chops from three classification of stress-susceptible pigs

| | SENSORY ATTRIBUTE | | | | |
|---|---|---|---|---|---|
| CLASS | INITIAL TENDERNESS | SUSTAINED TENDERNESS | INITIAL JUICINESS | SUSTAINED JUICINESS | FLAVOR |
| Negative | 71.7±1.6[a] | 74.0±1.7[a] | 78.7±1.7[a] | 79.5±1.7[a] | 75.4±1.6[a] |
| Carrier | 69.4±1.6[b] | 71.2±1.7[b] | 82.6±1.7[a] | 82.3±1.7[a] | 76.7±1.5[a] |
| Positive | 58.5±1.5[c] | 61.0±1.6[c] | 74.6±1.6[a] | 76.0±1.5[a] | 73.0±1.5[a] |

[z]0 = least intensity of juiciness, tenderness, or pork flavor; 150 = greatest intensity of juiciness, tenderness, or pork flavor.

[a,b,c]Means with different superscripts in the same column are significantly different at P<.05.

Table 3: Mean sensory values[z] of loin chops broiled to two end-point temperatures

| | SENSORY ATTRIBUTE | | | | |
|---|---|---|---|---|---|
| END POINT TEMPERATURE | INITIAL TENDERNESS | SUSTAINED TENDERNESS | INITIAL JUICINESS | SUSTAINED JUICINESS | FLAVOR |
| 71°C (160°F) | 73.3±1.4[a] | 75.6±1.4[a] | 83.3±1.4[a] | 84.0±1.4[a] | 75.4±1.2[a] |
| 77°C (170°F) | 59.5±1.2[b] | 62.1±1.3[b] | 74.2±1.3[b] | 74.5±1.3[b] | 74.5±1.3[a] |

[z]0 = least intensity of juiciness, tenderness, or pork flavor; 150 = greatest intensity of juiciness, tenderness, or pork flavor.

[a,b]Mean with different superscripts in the same column are significantly different at $P<.05$.

in juiciness when cooking to the higher end-point temperature, but tenderness scores were not different for the four end-point temperatures observed. They concluded from their data that the optimum end-point temperature was between 71.1°C and 76.°C. The results reported herein also indicate that a lower temperature of end-point doneness should be implemented to obtain the highest palatability of pork chops.

PST treatment did not significantly affect the proximate protein composition (Table 4) of raw pork chops. Fat composition was, however, significantly ($P<.05$) decreased by PST treatment and moisture was significantly increased (Table 4). Other

Table 4: Mean values for proximate composition of the intramusclular portion of loin chops from placebo (control) and porcine somatotropin supplemented pigs

| PST TREATMENT | % MOISTURE | % FAT | % PROTEIN |
|---|---|---|---|
| Placebo | $72.3\pm0.2^a$ | $3.5\pm0.2^a$ | $23.5\pm0.1^a$ |
| PST | $73.4\pm0.2^b$ | $2.4\pm0.2^b$ | $23.6\pm0.2^a$ |

$^{a,b}$Means with different superscripts in the same column are significantly different at $P<.05$.

researchers have also observed a decrease in lipid and increase in moisture in pigs treated with PST (Etherton et al., 1986; Prusa et al., 1989). No treatment interaction was observed in moisture and protein content. An interaction between PST and stress susceptibility was observed for fat and as expected genotypes

with greater intramuscular fat contents showed a greater reduction due to PST treatment than genotypes with lkower intramuscular fat contents (Table 5).

Table 5: Subclass means for lipid percentages of loin chops from placebo (control) and porcine somatotropin supplemented pigs of different stress susceptibilities

| GENOTYPE | PST TREATMENT | % FAT |
|---|---|---|
| Negative | Placebo | $3.5\pm0.4^{bc}$ |
| Negative | PST | $2.8\pm0.4^{ab}$ |
| Carrier | Placebo | $4.5\pm0.4^{c}$ |
| Carrier | PST | $2.2\pm0.4^{a}$ |
| Positive | Placebo | $2.5\pm0.4^{ab}$ |
| Positive | PST | $2.4\pm0.5^{a}$ |

$^{a,b,c}$Means with different superscripts in the same column are significantly different at $P<.05$.

Hunter L values of raw pork chops from animals treated with PST were significantly ($P<.05$) lower than values from control animals (Table 6). This means that the lean color of the chops from PST treated animals were darker than that of control chops. No effect was observed for Hunter a and b values (Table 6). No significant interaction between stress classification and PST treatment was observed. Stress classification significantly ($P<.05$) effected Hunter L values, but had no effect on Hunter a and b values (Table 7). That is, chops from stress positive animals had significantly ($P<.05$) higher Hunter L values

Table 6: Mean Hunter Lab values for loin chops from PST treated pigs

| TREATMENT | L | a | b |
|---|---|---|---|
| Placebo | 42.5±0.6[b] | 4.9±0.2[a] | 8.0±2.0[a] |
| PST | 39.4±0.6[a] | 5.0±0.2[a] | 10.1±2.1[a] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

Table 7: Mean Hunter Lab values for loin chops from three classifications of stress susceptible pigs

| CLASS | L | a | b |
|---|---|---|---|
| Negative | 38.7±0.8[a] | 4.8±0.3[a] | 11.4±2.5[a] |
| Carrier | 40.4±0.8[a] | 4.8±0.3[a] | 7.5±2.5[a] |
| Positive | 43.6±0.8[b] | 5.3±0.3[a] | 8.4±2.5[a] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

than chops from either stress negative or carrier animals. This means that the lean color of chops from the stress positive animals were lighter in color than the other chops. Ewan et al. (1979) and Swatland (1986) also observed increased reflectance values from chops of PSE animals. No lean color differences in our study were observed between the chops from negative or carrier animals.

Cook yields of loin chops was not significantly (P<.05) effected by stress classification or PST treatment when averaged across the different cooking conditions. Merkel (1971), however, observed increased cook losses from roasts that exhibited the PSE condition.

Furthermore, cooking to the higher (77°C) end-point temperature significantly (P<.05) lowered cook yields for pork chops (Table 8).

Table 8: Mean percent cook yield of pork chops broiled to two end-point temperatures

| TEMPERATURE | % COOK YIELD |
|---|---|
| 71°C (160°F) | 69.5±0.9[a] |
| 77°C (170°F) | 64.4±0.9[b] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

The compression of raw chops was conducted in an attempt to quantitate the softness of the muscle observed visually. The resistance to compression of raw pork chops was not significantly (p<.05) effected by PST treatment of animals, nor was there a significant interaction between PST treatment and stress classification. Stress classification, however, significantly (P<.05) affected resistance to compression. Chops from stress positive animals had a significantly (P<.05) higher resistance to compression than either chops from stress negative animals or carrier animals (Table 9). This is most likely due to greater compaciton of the muscle fibers in stress positive animals because of reduced interstitial water.

In summary, broiled pork chops from PST supplemented pigs had reduced sensory values for initial tenderness, initial and sustained juiciness and flavor. Chops from stress susceptible animals had

Table 9: Mean resistance to compression values of loin chops from animals of three classification of stress susceptible pigs

| CLASS | RESISTANCE (g/2.54 $cm^2$) |
|---|---|
| Negative | 719.6 + 409.6[a] |
| Carrier | 1211.0 + 396.6[a] |
| Positive | 2141.1 + 464.1[b] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

reduced initial and sustained tenderness values. Although PST reduced palatability attributes, the differences were small. There was no interaction between PST treatment and stress susceptibility of pigs for any of the measurements. These results suggest that PST treatment did not cause an increased incidence of PSE or similar muscle anomalies in stress susceptible animals. Pork chops broiled to an internal end-point of 71°C had greater initial and sustained tenderness and initial and sustained juiciness values than chops broiled to the end-point of 77°C. These results suggest that a lower temperature of end-point doneness should be implemented to obtain the highest palatability of broiled pork chops.

## Acknowledgements

We gratefully acknowledge the support of Pitman-Moore, Inc. and The National Livestock and Meat Board. We also thank Angie Nissen and Susan Joens for their technical support.

Journal Paper No.J-14065 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project 2711.

## REFERENCES

A.O.A.C. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D. C.

Allen, W. M., and Patterson, D. S. P. 1971. The possible relationship between plasma creatine phosphokinase activity and muscle characteristics in the pig. Pages 90-96 in J. C. M. Hasses-de Herr, G. R. Schmidt, W. Sybesma, and P. G. van der Wal, eds. Proc. of the 2nd International Symposium on Conditions and Meat Quality of Pigs. Centre of Agriculture Publishing and Documentation, Wageningen, The Netherlands.

Andersen, L. D., Parrish, Jr., F. C., and Topel, D. G. 1975. Histochemical and palatability properties of M. longissimus from stress-resistant and stress-susceptible porcine animals. J. Anim. Sci. 41:1600.

Cheah, K. S., Cheah, A. M., Crosland, A. R., Casey, J. C., and Webb, A. J. 1984. Relationship between $Ca^{2+}$ release, sarcoplasmic $Ca^{2+}$, glycolysis and meat quality in halothane-sensitive and halothane-insensitive pigs. Meat Sci. 10:117.

Christian, L. L. 1974. Halothane test for PSS-field application. Pages 6-13 in Proc. Am. Assn. Swine Practitioners Conf., Des Moines, Iowa.

Chung, C. S., Etherton, T. D. and J. P. Wiggins. 1985. Stimulation of swine growth by porcine growth hormone. J. Anim. Sci. 60:118.

Etherton, T. D., Wiggins, J. P., Chung, C. S., Evock, C. M., Rebhun, J. F., and Walton, P. E. 1986. Stimulation of pig growth performance by porcine growth hormone and growth hormone releasing factor. J. Anim. Sci. 63:1389.

Ewan, R. C., Topel, D. G., and Ono, K. 1979. Chemical composition of chops from pale, soft, exudative (PSE) and normal pork loins. J. Food Sci. 44:678.

Heymann, H., Hedrick, H. B., Karrasch, M. A., Eggeman, M. K., and Ellersieck, M. R. 1990. Sensory and chemical characteristics of fresh pork roasts cooked to different endpoint temperatures. J. Food Sci. 55:613.

Kanis, E., Vander Hel, W., Kouwenberg, W. J. A., Huisman, J., Politiek, R. D., Verstegen, M. W. A., Van der Wal, P.. And Van Weerden, E. J. 1988. Effects of recombinant porcine somatotropin (rPST) on meat quality of pigs. J. Anim. Sci. 66 (Suppl. 1):280.

Merkel, R. A. 1971. Processing and organoleptic properties of normal and PSE porcine muscle. Proc. Intl. Symp. Condition Meat Qual. 2:261.

Novakofski, J. E., McKeith, F. K., Grebner, G. L., McLaren, D. G., Brenner, M. K., Easter, R. A., Bechtel, P. J., Jones, R. W. and Ingle, D. L. 1988. Pig carcass and pork sensory characteristics for animals given daily injections of natural or recombinant porcine somatotropin from 57 to 108 kg. British Society of Animal Production Winter Meeting, Midlothian, Scotland.

Prusa, K. J., Love, J. A., and Miller, L. F. 1989. Composition and sensory analysis of rib chops from pigs supplemented with porcine somatotropin (pSt). J. Food Qual. 12:455.

Rasmusen, B. A. and Christian, L. L. 1976. H blood types in pigs as predictors of stress-susceptibility. Science 191:947.

SAS Institute Inc. 1985a. SAS User's Guide: Basics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

SAS Institute Inc. 1985b. SAS User's Guide: Statistics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

Solomon, M. B., Campbell, R. G., and Steele, N. C. 1990. Effect of sex and exogenous porcine somatotropin on longissimus muscle fiber characteristics of growing pigs. J. Anim. Sci. 68:1176.

Solomon, M. B., Campbell, R. G., Steele, N. C., and Caperna, T. J. 1989. Effects of exogenous porcine somatotropin administration between 30 and 60 kilograms on longissimus muscle fiber morphology and meat tenderness of pigs grown to 90 kilograms. J. Anim. Sci. 67(Suppl. 1):153 Abstr.

Swatland, H. J. 1986. Color measurements on pork and veal carcasses by fiber optic spectrophotometry. Can. Inst. Food Sci. Technol. J. 19:170.

Szczesniak, A. S. 1986. Sensory texture evaluation methodology. Proc. Recip. Meat Conf. 39:86.

Topel, D. G., Staun, H., and Riis, H. M. 1975. Relationships between stress adaptation traits in swine with skeletal muscle characteristics. World Rev. Anim. Prod. 10:52.

Warriss, P. D. 1982. The relationship between $pH_{45}$ and drip in pig muscle. J. Food. Technol. 17:573.

# SECTION II: PALATABILITY AND PROCESSING CHARACTERISTICS OF HAMS FROM PIGS OF THREE STRESS CLASSIFICATIONS TREATED WITH PORCINE SOMATOTROPIN

TITLE: Palatability and processing characteristics of hams from pigs of three stress classifications treated with porcine somatotropin

AUTHORS J. A. Boles, F. C. Parrish, Jr., C. L. Skaggs, and L. L. Christian

AFFILIATION: Departments of Animal Science and Food Technology, Iowa State University, Ames, IA 50011

RUNNING TITLE: Palatability of hams from PST treated pigs

## ABSTRACT

Forty-eight pigs of three known stress genotypes were injected daily with recombinant porcine somatotropin (PST) (4 mg/day) or placebo. *Semimembranosus* (SM) muscles from these animals were processed into hams, and sensory evaluation, color, proximate analysis and percent cook yields were done.

PST treatment of animals had no effect on the sensory scores, lipid or protein content, cook yields, or color values of hams. Hams from stress susceptible animals, however, had reduced sensory scores, smaller cook yields, and higher Hunter a and b values. Hams from stress susceptible animals also had a significantly smaller lipid content. No interaction between PST treatment and stress classification was observed for any of the measurements.

## INTRODUCTION

Consumer demand for lean meat has caused the pork industry to research new ways to produce leaner pork. Recent research has focused on the use of recombinant growth hormone (porcine somatotropin, PST) to increase growth rate, improve feed efficiency, and reduce carcass fat. Chung et al. (1985), for example, have observed decreased carcass fat and increased protein in animals treated with PST. The use of growth promotants can lead to increases in lean meat and more efficient production (Baile et al., 1983; Etherton et al., 1986). Some researchers, however, have seen an increase in the incidence of pale, soft, exudative (PSE) meat with the treatment of light weight animals with exogenous pituitary growth hormone (Solomon et al., 1989; Solomon et al., 1990). PSE muscle is usually associated with stress susceptible animals (Topel et al., 1975; Cheah et al., 1984) and leads to decreased processing yields (Merkel, 1971). Because of the suggested PSE problem along with the decreased fat, processors are concerned about the effect of porcine somatotropin on the processing and palatability characteristics of the muscle from these animals. Also few researchers have observed the effect of PST on the processing characteristics of hams. The objective of this study was to determine the effect of PST and three stress classifications on sensory and processing characteristics of hams.

## MATERIALS AND METHODS

Forty-eight crossbred pigs (24 barrows, 24 gilts) were allocated to six treatment groups consisting of eight head (4 barrows, 4 gilts)/treatment based on stress classification, and Pitman-Moore (PM) porcine somatotropin (PST) or placebo administration. The research trial consisted of two replicates based on availability of animals of the different stress classification genotypes within each litter. When possible, littermates of each stress classification were assigned to each treatment combination. Stress classification was determined by halothane screening (Christian, 1974), creatine phosphokinase (CPK) (Allen and Patterson, 1971), and blood typing (Rasmusen and Christian, 1976). Groups were designated as follows: stress-positive placebo (SP); stress-positive PST (SPPST); stress-carrier placebo (SC); stress-carrier PST (SCPST); stress-negative placebo (SN); stress-negative PST (SNPST). Each pig was injected in the neck once daily until taken off test. PST treatment was terminated at weekly intervals as individual pigs reached 109 lbs., but animals continued to be fed for 6 additional days for required withdrawal period. Pigs, after an overnight fast, were slaughtered at the Iowa State University Meat Laboratory. *Longissimus dorsi* (LD) samples were collected 45-min post-slaughter for determination of muscle pH (Warriss, 1982) to confirm stress classification. After a 24-hr postmortem chill (4°C) *semimembranosus* (SM) muscles were obtained from one side, vacuum-packaged and frozen at -29°C.

## Ham Production

SM muscles were thawed at 2°C for 48 hrs and were injected with brine to 25% of original weight. The brine contained 80% water, 11.0% salt, 6.6% sugar, 2.2% phosphate, 0.06% sodium nitrite and 0.22% sodium erythorbate. Raw and pumped weights of individual muscles were recorded. SM muscles were submerged in brine and allowed to equilibrate for 4 days and weights were again recorded. Then each muscle was stuffed into a casing and thermally processed and smoked in a AS Commander 625 thermal processing unit. The first step in the cooking process was 45 min on Hot Air with the dry bulb temperature at 82°C, then 15 min drying at 82°C. The next step was a 45-min hot smoke treatment without moisture, then 1 hr of hot smoking with the wet bulb set at 74°C. To finish, the product was cooked in hot air to an internal temperature of 63°C and was then cooked with moist heat (85°C) to a final internal temperature of 68°C. Final cooked weights were recorded, and hams were vacuum packaged and stored in a 2°C cooler until sensory evaluation, proximate analysis and color measurements were conducted.

## Sensory Evaluation

Sensory evaluation of ham slices was done by an inexperienced panel consisting of approximately 30 ISU faculty, staff and students. Ham slices (1.27 cm) were removed from the center of the SM muscle and cut into 1.27 cm cubes. Samples were served cold (4°C) and the panelists used a seven point facial hedonic scale to record their impressions (likes and dislikes). Numbers were assigned for statistical

analysis (7 = like extremely, 1 = dislike extremely). Sensory attributes evaluated were juiciness, texture, flavor and overall palatability. Means were used for statistical analysis.

## Proximate Analysis

Moisture, fat and protein content of cured and cooked ham slices were done by following AOAC (AOAC, 1984) procedures used in the ISU Meat Laboratory.

## Color Measurements

Color measurements for processed ham slices were conducted by using a Hunter Labscan with a fluorescent light source and a 1.27 cm aperture opening. Measurments were made on samples overwrapped with polyvinylchloride wrap. The mean of three measurements was used for statistical analysis.

## Statistics

The design of this study was a 2x3 factorial design. The two factors of the experimental design were two PST treatments and three stress genotype classifications. Analysis of all data was performed by using the Statistical Analysis System (SAS Institute Inc., Cary N. C.). The general linear model procedure was used; guidance for model statements tests of significance, was obtained from SAS Institute Inc. (1985a, b).

## RESULTS AND DISCUSSION

PST treatment of animals had no significant ($P<.05$) effect on the sensory attributes of ham slices, nor was an interaction between the stress classification and PST observed. These observations agree with data published by Prusa and co-workers (1990) who reported that trained panelists found no difference in sensory attributes of hams from animals treated with PST (4 mg/day). Ham slices from stress positive animals, however, had significantly ($P<.05$) decreased scores for texture, flavor and overall palatability (Table 1). This agrees with observations of Wismer-Pedersen (1960). He found lowered taste and texture scores for hams made from PSE muscle.

PST treatment of animals had no significant effect on protein and lipid composition of cooked hams, but significantly ($P<.05$) increased moisture content (Table 2). Prusa and co-workers (1989), however, saw no difference in fat, moisture, or protein content in cooked SM muscles from pigs treated with PST (4 mg/day). Hams from stress positive animals had a significantly ($P<.05$) less lipid content than normal animals, but was no different from carrier animals (Table 3). Hams from stress positive animals were not different in moisture or protein content. Merkel (1971) also observed no difference in proximate composition between hams made form normal or PSE muscle. No interaction between PST treatment and stress classification was observed in this study for moisture lipid or protein contents.

Table 1: Mean sensory values[z] for hams from animals of three classification of stress susceptible pigs

| | SENSORY ATTRIBUTE | | | |
|---|---|---|---|---|
| CLASS | JUICINESS | TEXTURE | FLAVOR | OVERALL PALATABILITY |
| Normal | 5.0± 0.2[a] | 4.9±0.1[ab] | 5.1±0.1[a] | 5.0±0.1[a] |
| Carrier | 5.1± 0.1[a] | 5.1±0.1[a] | 5.1±0.1[a] | 5.1±0.1[a] |
| Positive | 4.7±0.2[a] | 4.6±0.1[b] | 4.7±0.1[b] | 4.6±0.1[b] |

[z]Scores were assigned to ratings on a 7 point facial hedonic scale, 7 was liked the most and 1 the least.

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

Table 2: Mean values for proximate composition of hams from PST treated pigs

| TREATMENT | %MOISTURE | %LIPID | %PROTEIN |
|---|---|---|---|
| Placebo | 70.2±0.3[b] | 2.5±0.2[a] | 21.8±0.6[a] |
| PST | 71.2±0.3[a] | 2.0±0.2[a] | 20.7±0.6[a] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

Table 3: Mean values for proximate composition of hams from animals of three classifications of stress susceptible pigs

| CLASS | %MOISTURE | %LIPID | %PROTEIN |
|---|---|---|---|
| Normal | 70.5±0.3[a] | 3.0±0.3[a] | 21.7±0.7[a] |
| Carrier | 71.3±0.3[a] | 2.2±0.3[b] | 20.4±0.7[a] |
| Positive | 70.4±0.3[a] | 1.7±0.3[b] | 21.6±0.7[a] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

PST treatment of animals did not significantly (P<.05) affect Hunter values of ham slices nor was there a significant (P<.05) interaction between PST and stress classification. Ham slices from stress positive animals, however, had significantly (P<.05) greater Hunter a and b (Table 4) values. These values would indicate a redder and yellower color in hams made from PSE muscle than hams made from normal muscle. Stress classification of animals did not significantly (P<.05) influence Hunter L values of ham slices. This

observation disagrees with data from Merkel (1971) in which he found a paler color in hams made from PSE muscle.

Table 4: Mean Hunter Lab values for hams from animals of three classification of stress susceptible pigs

| CLASS | L | a | b |
|---|---|---|---|
| Normal | 54.5±0.7[a] | 6.4±0.2[a] | 5.4±0.3[a] |
| Carrier | 53.4±0.6[a] | 6.6±0.2[a] | 5.4±0.2[a] |
| Positive | 54.9±0.7[a] | 7.3±0.2[b] | 6.2±0.3[b] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

Cooked yields and brine uptake of hams from PST treated animals were not significantly (P<.05) different from placebo animals. Hams from stress positive animals, however, had significantly (P<.05) smaller cook yields than hams from normal or carrier animals (Table 5). Merkel (1971) also showed a decrease in ham cook yields when the starting muscle displayed the PSE condition. No significant (P<.05) difference in brine uptake between the stress classifications was observed.

Table 5: Cooked yields for hams from animals of three classifications of stress susceptible pigs

| CLASS | %YIELD |
|---|---|
| Normal | 104.3±1.2[a] |
| Carrier | 104.5±1.2[a] |
| Positive | 99.9±1.3[b] |

[a,b]Means with different superscripts in the same column are significantly different at P<.05.

In summary, sensory scores for hams from PST treated animals were no different from control animals, nor was there any difference in processing characteristics. Consequently, the use of PST offers promise in reducing fatness and increasing leanness. These results suggest that PST has no detrimental effect on palatability or processing characteristics of hams, nor was there an increased incidence of PSE due to treatment of stress susceptible animals with PST. Those animals classified as stress susceptible, however, had lowered sensory scores and processing yields.

Acknowledgements

We gratefully acknowledge the support of Pitman-Moore, Inc. and The National Livestock and Meat Board. We also thank Angie Nissen and Susan Joens for their technical assistance.

Journal Paper No. J-14066 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, Project 2711.

## REFERENCES

A.O.A.C. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D. C.

Allen, W. M., and Patterson, D. S. P. 1971. The possible relationship between plasma creatine phosphokinase activity and muscle characteristics in the pig. Pages 90-96 in J. C. M. Hasses-de Herr, G. R. Schmidt, W. Sybesma, and P. G. van der Wal, eds. Proc. of the 2nd International Symposium on Conditions and Meat Quality of Pigs. Centre of Agriculture Publishing and Documentation, Wageningen, The Netherlands.

Baile, C. A., Della-Fera, M. A., and McLaughlin, C. L. 1983. Performance and carcass quality of swine injected daily with bacterially-synthesized human growth hormone. Growth 47:225.

Cheah, K. S., Cheah, A. M., Crosland, A. R., Casey, J. C., and Webb, A. J. 1984. Relationship between $Ca^{2+}$ release, sarcoplasmic $Ca^{2+}$, glycolysis and meat quality in halothane-sensitive and halothane-insensitive pigs. Meat Sci. 10:117.

Christian, L. L. 1974. Halothane test for PSS-field application. Pages 6-13 in Proc. Am. Assn. Swine Practitioners Conf., Des Moines, Iowa.

Chung, C. S., Etherton, T. D. and J. P. Wiggins. 1985. Stimulation of swine growth by porcine growth hormone. J. Anim. Sci. 60:118.

Etherton, T. D., Wiggins, J. P., Chung, C. S., Evock, C. M., Rebhun, J. F., and Walton, P. E. 1986. Stimulation of pig growth performance by porcine growth hormone and growth hormone releasing factor. J. Anim. Sci. 63:1389.

Kanis, E., Vander Hel, W., Kouwenberg, W. J. A., Huisman, J., Politiek, R. D., Verstegen, M. W. A., Van der Wal, P.. And Van Weerden, E. J. 1988. Effects of recombinant porcine somatotropin (rPST) on meat quality of pigs. J. Anim. Sci. 66 (Suppl. 1):280.

Merkel, R. A. 1971. Processing and organoleptic properties of normal and PSE porcine muscle. Proc. Intl. Symp. Condition Meat Qual. 2:261.

Prusa, K. J., Love, J. A., and Miller, L. F. 1989. Composition, water-holding capacity and pH of muscles from pigs supplemented with porcine somatotropin (pST). J. Food Qual. 12:467.

Prusa, K. J., Sebranek, J. G., Love, J. A., and Miller, L. F. 1990. Quality characteristics of Canadian-style bacon, boneless hams and bacon processed from pigs supplemented with porcine somatotropin. J. Food Sci. In Press.

Rasmusen, B. A. and Christian, L. L. 1976. H blood types in pigs as predictors of stress-susceptibility. Science 191:947.

SAS Institute Inc. 1985a. SAS User's Guide: Basics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

SAS Institute Inc. 1985b. SAS User's Guide: Statistics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

Solomon, M. B., Campbell, R. G., and Steele, N. C. 1990. Effect of sex and exogenous porcine somatotropin on longissimus muscle fiber characteristics of growing pigs. J. Anim. Sci. 68:1176.

Solomon, M. B., Campbell, R. G., Stelle, N. C., and Caperna, T. J. 1989. Effects of exogenous porcine somatotropin administration between 30 and 60 kilograms on longissimus muscle fiber morphology and meat tenderness of pigs grown to 90 kilograms. J. Anim. Sci. 67(Suppl. 1):153 Abstr.

Topel, D. G., Staun, H., and Riis, H. M. 1975. Relationships between stress adaptation traits in swine with skeletal muscle characteristics. World Rev. Anim. Prod. 10:52.

Warriss, P. D. 1982. The relationship between $pH_{45}$ and drip in pig muscle. J. Food. Technol. 17:573.

Wismer-Pedersen, J. 1960. Effect of cure on pork with watery structure. I. Binding of salt and water to the meat. Food Res. 25:789.

# SECTION III: EFFECT OF PORCINE STRESS SYNDROME ON THE SOLUBILITY AND DEGRADATION OF MYOFIBRILLAR/CYTOSKELETAL PROTEINS

TITLE: Effect of porcine stress syndrome on the solubility and degradation of myofibrillar/cytoskeletal proteins.

AUTHORS: J. A. Boles, F. C. Parrish, Jr., T. W. Huiatt, and R. M. Robson

AFFILIATION: Muscle Biology/Meat Science, Meat Export Research Center, Iowa State University, Ames, IA 50011

RUNNING TITLE: Protein solubility and degradation of PSE pork

## ABSTRACT

*Longissimus dorsi* muscle samples were obtained from each of 6 stress positive, 6 stress negative and 5 stress carrier animals 45 minutes post-slaughter and analyzed on 1, 3, 5, and 7 days postmortem. Purified myofibrils were prepared for gel electrophoresis, and muscle samples were extracted with phosphate buffers containing KCl or KI. Samples for SDS-PAGE were made from each extraction. Sarcoplasmic and myofibrillar/cytoskeletal protein solubility was significantly lower in muscle samples from stress positive animals. Postmortem degradation of titin was different in myofibrils purified from stress positive animals in that no increase in intensity of $T_2$ bands at days 5 and 7 was observed. The combination of reduced solubility and degradation of structural constraints may explain the reduced WHC and functionality of muscle from stress susceptible animals.

## INTRODUCTION

Porcine stress syndrome (PSS) has been recognized as a problem in the pork industry since the late 50s-early 60s. Pale, soft, exudative (PSE) muscle is usually associated with stress susceptible animals (Topel et al., 1975; Cheah et al., 1984). These stress animals show an abnormal calcium ion homeostasis causing a flood of calcium into the system upon stress, activating muscle contraction and glycolysis (Greaser et al., 1969; Cheah et al., 1984; Ludvigsen, 1985; Topel and Hallberg, 1985). PSE muscle also displays a rapid postmortem pH decline because of increased glycolysis (Eikelenboom and van den Bergh, 1973; Warriss, 1982), a reduction in water-holding capacity (Penney, 1977; Warriss, 1982) and decreased protein solubility (Bendall and Wismer-Pedersen, 1962; Sayre and Briskey, 1963), along with a pale, unstable color (McLoughlin and Goldspink, 1963; Topel et al., 1976; Jensen and Barton-Gade, 1985; Swatland, 1986). This condition leads to a loss of functionality of the proteins in processed products and large weight losses in both fresh and processed products. Bendall and Wismer-Pedersen (1962) observed a marked decrease in solubility of the proteins in high salt buffers, but saw no difference in the isoelectric point of these proteins. They concluded from their studies that myofibrils from PSE muscle were not denatured or aggregated in the usual sense, but were probably covered by a layer of denatured sarcoplasmic protein that was firmly bound to the surface of the myofibrils.

Hamm (1960) concluded that the water-holding capacity of muscle was mostly affected by the charge on the protein caused by changes in pH. Offer and Trinick (1983) later observed that structural constraints within the myofibril, Z and M lines, and myosin cross-bridges, also affected the water-holding capacity of muscle. The removal of these structural constraints, by using salt and phosphates, resulted in increased water-holding capacity. Paterson and co-workers (1988) observed an increase in the extraction of titin with salt and phosphate solutions and a concomitant increase in water-holding capacity, suggesting that the removal of titin from structural constraints, like gap filaments, may be of consequence in improving water-holding capacity. PSE muscle displays markedly reduced water-holding capacity (Bendall and Wismer-Pedersen, 1962; Sayre and Briskey, 1963; Warriss, 1982), suggesting that either pH or structural constraints, or both, are altered in PSE muscle.

Calpain is a protease located within the muscle, and is activated by calcium with a pH optima of 7.0-7.4 (Dayton et al., 1974). Significant activity is evident between 6.5 and 8.0, but calpain activity decreases rapidly below pH 6.5 and above 8.0 (Dayton et al., 1976). Proteolysis by calpain is very limited and specific (Olson et al., 1977). Indeed a 30,000 dalton component found in tender muscle is the result of calpain and postmortem tenderization (Olson et al., 1977; MacBride and Parrish, 1977). Digestion of purified myofibrils with calpain results in the

removal of Z-lines and the release of intact α-actinin (Reddy et al., 1974). Calpain also degrades purified tropomyosin, troponin-I, and troponin-T, but does not effect myosin and actin (Dayton et al., 1974). Lusby and co-workers (1983) observed titin disappearance with postmortem aging. This postmortem disappearance of titin is dependent on the presence of calcium (Takahashi and Saito, 1979), suggesting degradation of the protein by calpain. The flood of calcium into the PSE muscle would suggest an increased proteolysis; however, Kang and co-workers (1978) observed that myofibrils from PSE muscle were more resistant to calpain digestion than myofibrils from normal muscle. Consequently, a number of questions about the fate of myofibrillar/cytoskeletal proteins during postmortem storage of pork muscle remain unanswered. The objective of this study was to determine the effect of PSE on the solubility of sarcoplasmic and myofibrillar/cytoskeletal proteins, and the effect of this condition on the postmortem degradation of these proteins.

## MATERIALS AND METHODS

Six stress negative, 5 stress carrier and 6 stress negative animals were slaughtered at the Iowa State Meat Laboratory. Stress classification of animals was determined by halothane screening (Christian, 1974), creatine phosphokinase (CPK) activity assays (Allen and Patterson, 1971), and blood typing for halothane reactive associated gene products (Rasmusen and Christian, 1976). *Longissimus dorsi* muscle samples were obtained 45 min post-slaughter and placed directly on ice. Samples for pH measurements were also obtained at 45 min to confirm PSE classification (Warriss, 1982). Muscle samples were stored at 2°C in Whirl-Pak™ bags for 0, 1, 3, 5, and 7 days post-slaughter and were then placed in a -70°C freezer until analysis.

### Myofibril Isolation

Myofibrils were prepared by a modification of the procedure of Lusby et al. (1983). After purification, myofibrils were washed twice with 10 volumes of 5 mM Tris-hydrochloride (pH 8.0) and pelleted by centrifugation at 3050 x g (Wang, 1982). The pellet was resuspended in 4 volumes of 5 mM Tris-hydrochloride and protein content determined by a modified biuret procedure (Robson et al., 1968). Samples for SDS-PAGE were prepared to contain 5 mg/mL of protein. One mL of the protein solution was heated at 50°C for 20 min. in 0.5 mL of

30 mM Tris-hydrochloride (pH 8.0), 3 mM EDTA, 3% (w/v) SDS, 7.5%(v/v) 2-mercaptoethanol, 30% (v/v) glycerol, and 0.3% (w/v) pyronin Y (Wang, 1982).

Protein Solubility

Protein solubility was determined following the procedures of Chaudhry et al. (1969). Quadruplicate samples of 4 g each were weighed into 50-mL centrifuge tubes and exact weights were recorded. Samples were homogenized for 10 sec in 10 volumes of 0.03 M $KPO_4$ (pH 7.4) and placed on a shaker in a 2°C cold room for 2 hrs. After 2 hrs, the sample was pelleted by centrifugation at 1400 x g for 20 min. The supernatant was decanted and saved for protein analysis and the pellet was resuspended in 10 volumes of 0.03 M $KPO_4$ by using a plastic spatula. The tubes were placed on the shaker for another 2 hrs. Protein content of the supernatant was determined by following the biuret protein determination procedure of Robson et al. (1968). SDS-PAGE samples were prepared from the supernatant to contain 4 mg/mL of protein, and were heated at 50°C for 20 min. in 7.5% (v/v) 2-mercaptoethanol, 1.5% (w/v) SDS, 15% sucrose, 0.05% (w/v) bromphenol blue, and 15 mM 2-N-morpholinoethanesulfonic acid (pH 6.5) (tracking dye) (Paterson and Parrish, 1987). After the second extraction with 0.03 M $KPO_4$, the insoluble protein was pelleted by centrifugation at 1400 x g for 20 min. Protein content in the supernatant was determined. The pellet in two tubes were resuspended in 0.5 M

KCl in 0.1 M phosphate buffer (pH 7.4). The other two pellets were resuspended in 1.1 M KI in 0.1 M phosphate buffer (pH 7.4). All of the tubes were returned to the shaker in the cold room (2°C) for 3 hrs. Insoluble protein was again pelleted by centrifugation at 1400 x g for 20 min. Protein concentrations in the supernatant and gel samples were prepared as described previously. Pelleted protein was resuspended in 0.5 M KCl or 1.1 M KI and placed on the shaker overnight. Insoluble protein was pelleted by centrifugation at 1400 x g for 20 min and protein determination of the supernatant was done as previously described. Gel samples were made from the insoluble protein by following the procedure of Fritz et al. (1989). One gram of the pellet was resuspended in buffer containing 75 mM potassium chloride, 10 mM potassium phosphate, 2 mM magnesium chloride, 2 mM EGTA, pH 7.0 to give approximately 3 mg/mL by using teflon homogenizers. Biuret protein determination was used to approximate protein in solution. The 3 mg/mL protein solution was mixed 1:1 with sample buffer which contained 8 M urea, 2 M thiourea, 3% (w/v) SDS, 0.7 M 2-mercaptoethanol, and 25 mM Tris-HCl at pH 6.8. SDS-PAGE samples were prepared to contain 1 mg/mL of protein and were heated at 50°C for 20 min. in tracking dye.

### Whole Muscle Samples

Whole muscle samples were prepared as described by the procedure of Bechtel and Parrish (1983). Muscle (0.5 g) samples

were scissor-minced, homogenized (Kontes glass homogenizer) in 14.5 mL of SDS buffer (2% (w/v) SDS, 10 mM sodium phosphate, pH 7.0) and then centrifuged at 1500 x g for 10 min. to remove undissolved material. Protein concentrations of the supernatants were determined by the biuret method, and gel samples were prepared as described previously.

SDS Polyacrylamide Gel Electrophoresis

SDS-PAGE was done according to the procedure of Laemmli (1970) with modifications to accommodate the different molecular weight proteins. The amount of protein loaded onto each gel was 40 μg for myofibrils and for soluble protein fractions. Gels containing 4% (w/v) (stacking gel) and 10% (separating gel) (w/v) acrylamide were prepared from a stock solution of 30% (w/v) acrylamide (37:1, acrylamide:N,N'-bis-methylene acrylamide). The final concentrations in the separation gels were as follows: 0.375 M Tris-HCl (pH 8.0), 0.1% SDS, and 2 mM EDTA. The gels were polymerized chemically by the addition of 0.025% tetramethylethlenediamine (TEMED) and ammonium persulfate. The 4% acrylamide stacking gel contained 0.375 M Tris-HCl (pH 6.7), 0.1% SDS and 2 mM EDTA. Changes in titin and nebulin were monitored using a 5% acrylamide (100 acrylamide:1 N,N'-bis-methylene acrylamide) gel containing 0.375 M Tris-HCl (pH 8.0), 0.1% SDS, and 2 mM EDTA. The dimensions of the gels were 160 mm x 180 mm. Gels were run at 35 mA. Gels were stained overnight in a 0.1% (w/v) Coomasie brilliant blue R-250, 10% glacial acetic acid, and 50% methanol, and then were destained in an excess of the same solution,

excluding the Coomasie brilliant blue. Sigma high molecular standards were used on 10% gels to aid in identification of proteins. The standard contained rabbit myosin, heavy chain (205 kDa), b- galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). Purified bovine titin and nebulin was used on 5% gels to identify proteins.

## Statistics

This study was a split-plot design. The whole plot was the animals of three different stress classifications and the split plot was the time of storage. Analysis of all data was performed by using the Statistical Analysis System (SAS Institute Inc., Cary N. C.). The general linear model procedure was used; guidance for model statements tests of significance was obtained from SAS Institute Inc. (1985a, b).

## RESULTS AND DISCUSSION

Results of protein solubility measurements on muscles from stress, carrier, and control pigs are compared in Figure 1. No interaction was observed between stress classification and time of storage postmortem; thus the data shown is average of all postmortem times. Muscle from stress positive animals had significantly ($P<0.05$) less soluble sarcoplasmic and myofibrillar/cytoskeletal proteins than either muscles from stress carrier or stress negative animals (Figure 1). These data agree with those of Bendall and Wismer-Pedersen (1962),

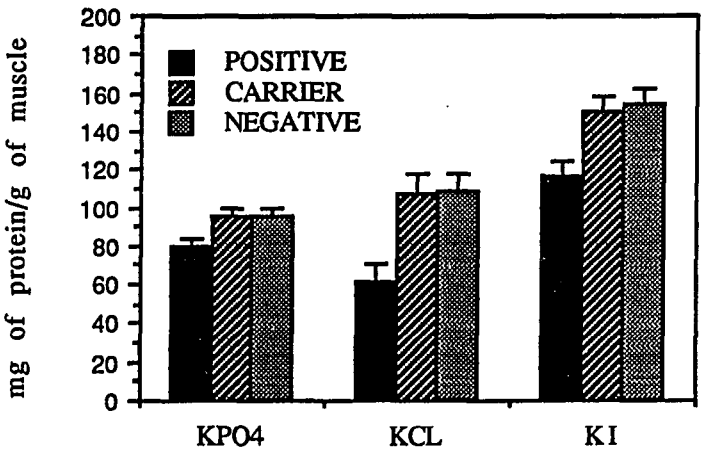



Figure 1: Sarcoplasmic and myofibrillar protein solubility of *longissimus* muscle from porcine animals of three stress classifications.

who reported a reduction in protein of high salt extracts from PSE muscle. Sayre and Briskey (1963) also found a reduction in the solubility of sarcoplasmic proteins when PSE muscle was extracted with water. Bendall and Wismer-Pedersen (1962) suggested that the decreased solubility was due to precipitation of the sarcoplasmic proteins onto the myofibril.

Time of postmortem storage at 2°C had a significant ($P<0.05$) effect on the solubility of the proteins among the days observed, but no apparent trend upward or downward was identified (Figure 2). The amount of protein extracted was similar to Chaudhry et al. (1969) who looked at the effect of postmortem time and storage temperature on the solubility of

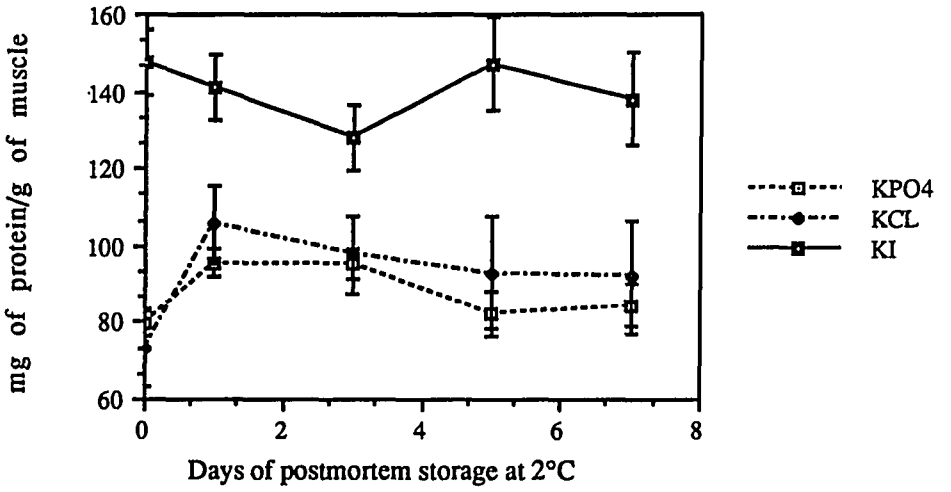
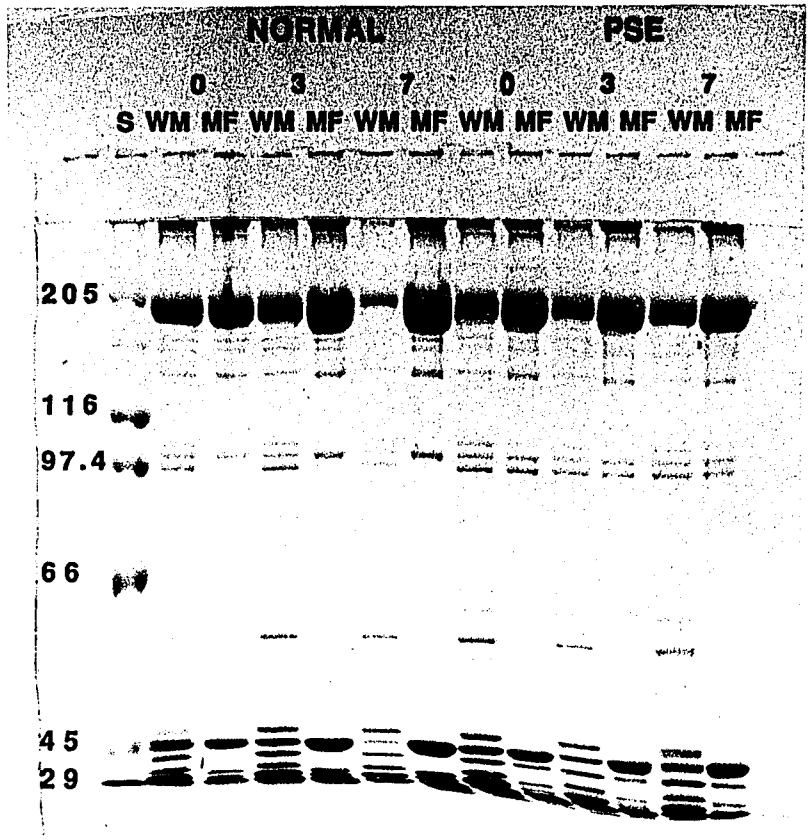



Figure 2: Effect of postmortem storage at 2°C on protein solubility of pork *longissimus* muscle from three stress classifications

rabbit and bovine muscle. They, however, found an increase in solubility of sarcoplasmic and myofibrillar proteins as time postmortem increased. Valin (1968) also determined the solubility of proteins in bovine muscle and saw an increase in protein solubility with increasing time postmortem. This discrepancy in the results reported herein could be due to the differences in solubility of proteins from muscle of stress susceptible animals.

Figure 3 shows a ten percent SDS-PAG electrophoretogram of whole muscle samples and isolated myofibrils from *longissimus* muscle 0, 3 and 7 days postmortem. Lanes that contained whole muscle samples displayed more protein bands than lanes that contain myofibrils because of the presence of both sarcoplasmic and myofibrillar proteins in the whole muscle extract. Also, whole muscle extracts from both normal and PSE muscle showed less intense bands of myosin (205 kDa) and actin (45 kDa) on SDS-PAG electrophoretograms than did those from myofibrils. Because the protein load in the lane is based on total protein content of the sample and whole muscle samples contain a larger variety of proteins, actin and myosin will be proportionately lower in the whole muscle samples, giving rise to less intense bands on SDS-PAGE. Whole muscle extracts from 7 days postmortem showed consistently less intense bands for all

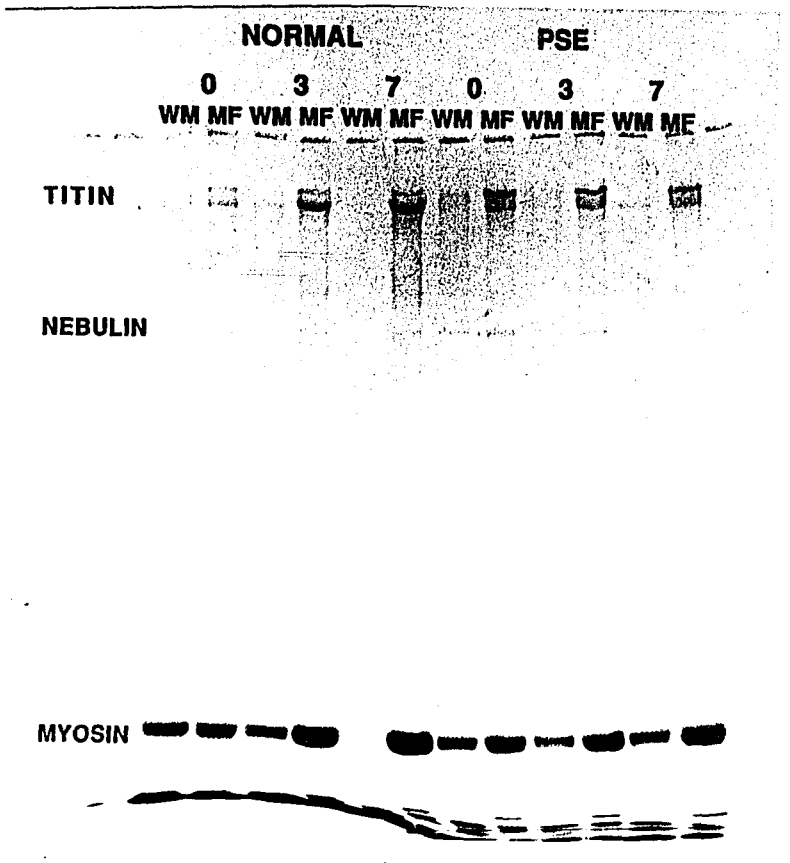
Figure 3: Ten percent SDS-PAG electrophoretogram of whole muscle homogenates and myofibrils (MF) from normal and stress positive (PSE) animals at 0, 3, and 7 days postmortem (S = Sigma high molecular weight standards) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa)


NORMAL
PSE
0
3
7
0
3
7
S WM MF WM MF WM MF WM MF WM MF WM MF
205
116
97.4
66
45
29

proteins. Intensity of α-actinin (100 kDa) bands in myofibrils isolated from normal muscle increased on days 3 and 7. In addition, actin (45 kDa) bands were less intense in myofibrils isolated from PSE muscle than in myofibrils isolated from normal muscle. Two extra bands, one above and one below α-actinin (100 kDa), are observed on SDS-PAG electrophoretograms in myofibrils isolated from PSE muscle, but are not observed in myofibrils isolated from normal muscle. Conversely, these bands are seen in whole muscle extracts from both PSE and normal muscle. The migration of one of these bands is similar to the phosphorylase b in the standard, suggesting that this protein is phosphorylase b. This band arise from adherence of sarcoplasmic proteins to the myofibril. The observation that these bands remain during isolation of myofibrils suggests that the adhesion of sarcoplasmic proteins to myofibrils is relatively strong and not broken with dilute salt. These results support the theory of Bendall and Wismer-Pedersen (1962), who suggested that the reduced protein solubility in PSE muscle was due to sarcoplasmic proteins precipitating onto the myofibril.

Figure 4 shows a 5% SDS-PAG electrophoretogram of whole muscle extracts and myofibrils from normal and PSE muscle. Titin bands ($T_1$ and $T_2$) were more intense in myofibrils than in whole muscle extracts, again due to the fact that equal protein loads were applied to all lanes. The loss of intensity of $T_1$ and an increase in the intensity of $T_2$ on day 7 suggests that titin

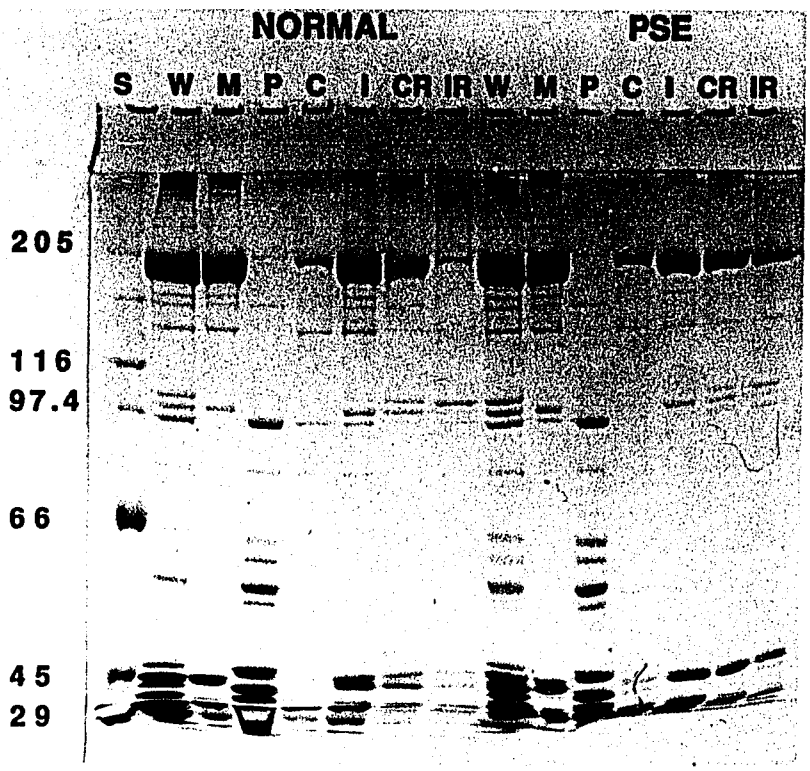
Figure 4: Five percent SDS-PAG electrophoretogram of myofibrils (MF) and whole muscle homogenates (WM) from normal and stress positive (PSE) animals at 0, 3, and 7 days postmortem (40 μg of total protein was loaded on each lane)


NORMAL
PSE
0
3
7
0
3
7
WM MF WM MF WM MF WM MF WM MF WM MF
TITIN
NEBULIN
MYOSIN

degradation occurred in myofibrils isolated from normal muscle. Myofibrils isolated from PSE, on the other hand showed the presence of the doublet and no increase in intensity of $T_2$ during postmortem storage. Nebulin band intensity also decreased in whole muscle samples from normal muscle, but no decrease in nebulin intensity was observed in myofibrils isolated from PSE muscle. These observations suggest the possibility that less proteolysis takes place in PSE muscle.

In general, the proteins from both normal and PSE were extracted similarly with buffered extracting solutions. The SDS-PAGE band locations showed that the same muscle proteins were extracted with each buffered salt solution from both PSE and normal muscle. Based on SDS-PAGE band location and intensity the 0.03 M phosphate buffer extracted a slight amount of myosin, and phosphorylase b and other sarcoplasmic proteins (Figure 5). Bands of sarcoplasmic proteins are more in number in whole muscle extracts than that found in myofibrils. Potassium chloride extracted fewer different proteins than did the potassium iodide solutions. This was not totally unexpected since the potassium iodide solution had a greater ionic strength, and because potassium iodide dissociates F-actin to G-actin (Szent-Gyorgyi, 1951) allowing a more complete extraction of proteins in the myofibril. Potassium iodide extracted M-protein (165 kDa), myomesin (185 kDa), α-actinin (100 kDa) and actin (45 kDa) more completely than did potassium chloride. Phosphorylase b

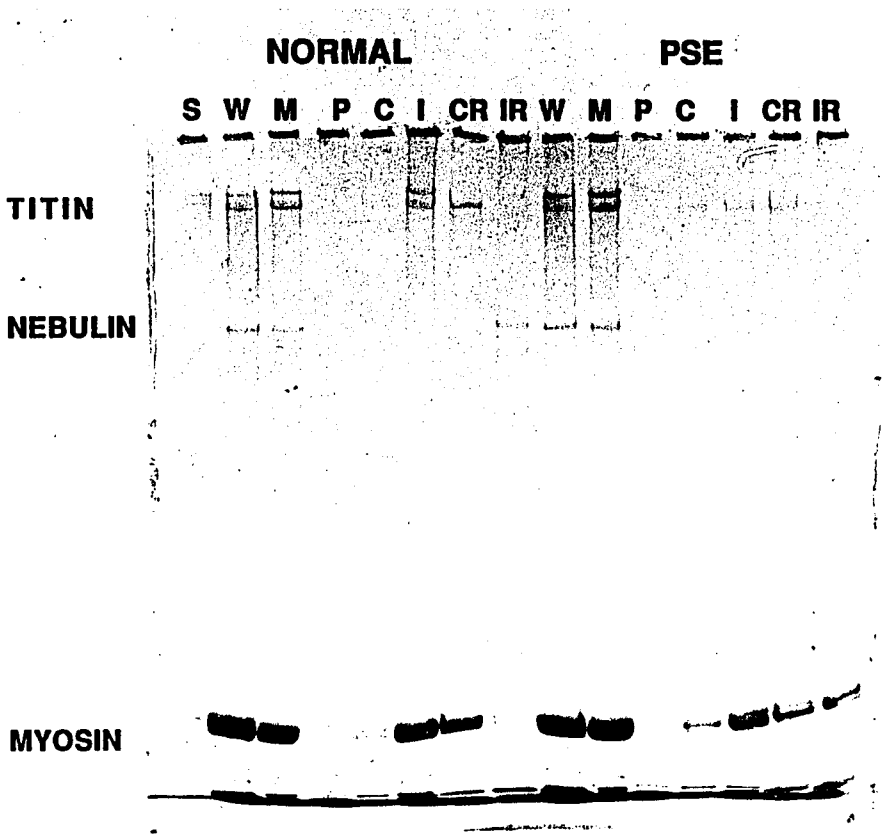
Figure 5: Ten percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 0 days postmortem from normal and PSE muscle (S=Sigma high molecular weight standard, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa)


NORMAL
PSE
S W M P C I CR IR W M P C I CR IR
205
116
97.4
66
45
29

(97.4 kDa) and C-protein (120 kDa), however, were extracted similarly with both buffers. More myosin was observed in the potassium chloride residue than the potassium residue, indicating less extraction of myosin by potassium chloride. Furthermore, a band at approximately 105 kDa was observed in the potassium iodide residue and the potassium chloride residue, but a more intense band was seen in both residues than in the soluble portions from both PSE and normal muscle. This suggests that this band at approximately 105 kDa is very insoluble. More phosphorylase b was observed in myofibrils isolated from PSE muscle than in myofibrils isolated from normal animals. The sarcoplasmic proteins (0.03 M $KPO_4$ extract) showed no difference in the band intensities between normal and PSE muscle (Figure 5). More proteins (myosin, C-protein, α-actinin, phosphorylase b, actin) were observed in the residues from PSE muscle than from normal muscle, supporting the observation that proteins from PSE are more insoluble.

Figure 6 shows a five percent SDS-PAG electrophoretogram of samples extracted with potassium iodide and potassium chloride of at-death (day 0) longisimus muscle from PSE and normal animals. In general, titin and nebulin are not extracted with 0.03 M potassium phosphate or 0.5 M potassium chloride. This agrees with Wang and co-workers (1979) who observed that titin was a very insoluble protein. Titin doublets, but not nebulin bands, were observed when muscle was extracted with potassium

Figure 6: Five percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 0 days postmortem from normal and PSE muscle (S=bovine titin, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane)


NORMAL
PSE
S W M P C I CR IR W M P C I CR IR
TITIN
NEBULIN
MYOSIN

iodide. Nebulin, as observed in the IR lane, remains in the potassium iodide residue. In addition, some $T_2$ is extracted with potassium chloride. More titin was extracted with potassium iodide and potassium chloride from normal muscle than from PSE muscle. No nebulin was evident in potassium chloride and potassium iodide extracts or residues from PSE muscle.

After 7 days of postmortem aging, the same general pattern of extraction was observed (Figures 7 and 8) as that observed in at-death muscle. However, based on protein band intensity, more of the 105 kDa protein, α-actinin and phosphorylase b was observed in potassium chloride extract from 7 day aged PSE muscle than from 0 day aged PSE muscle (Figures 5 and 7). Furthermore, more sarcoplasmic protein bands, between 205 and 116 kDa, were observed in potassium phosphate extracts from normal muscle on day 7 than on day 0. Furthermore, on day 7, $T_2$ was extracted with all buffered salt solutions (potassium phosphate, potassium chloride and potassium iodide) from normal muscle (Figure 8). In PSE muscle, however, $T_2$ was extracted only with potassium chloride and potassium iodide, but the bands were not as intense as those in normal muscle. In addition, myofibrils isolated from PSE showed strong doublets on gels after 7 days of aging, while normal muscle showed only $T_2$ bands. These observations suggest that whatever alters the solubility of the sarcoplasmic and myofibrillar/cytoskeletal proteins affects all of the proteins similarly. This agrees with

Figure 7: Ten percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 7 days postmortem from normal and PSE muscle (S=Sigma high molecular weight standards, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa)

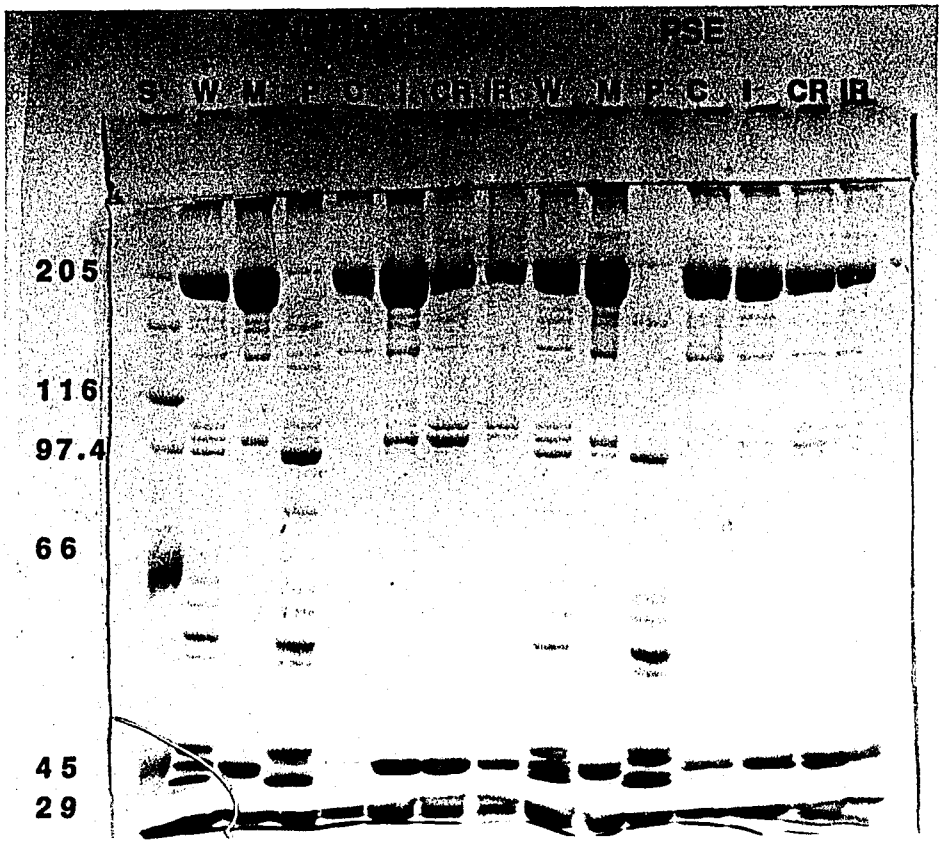
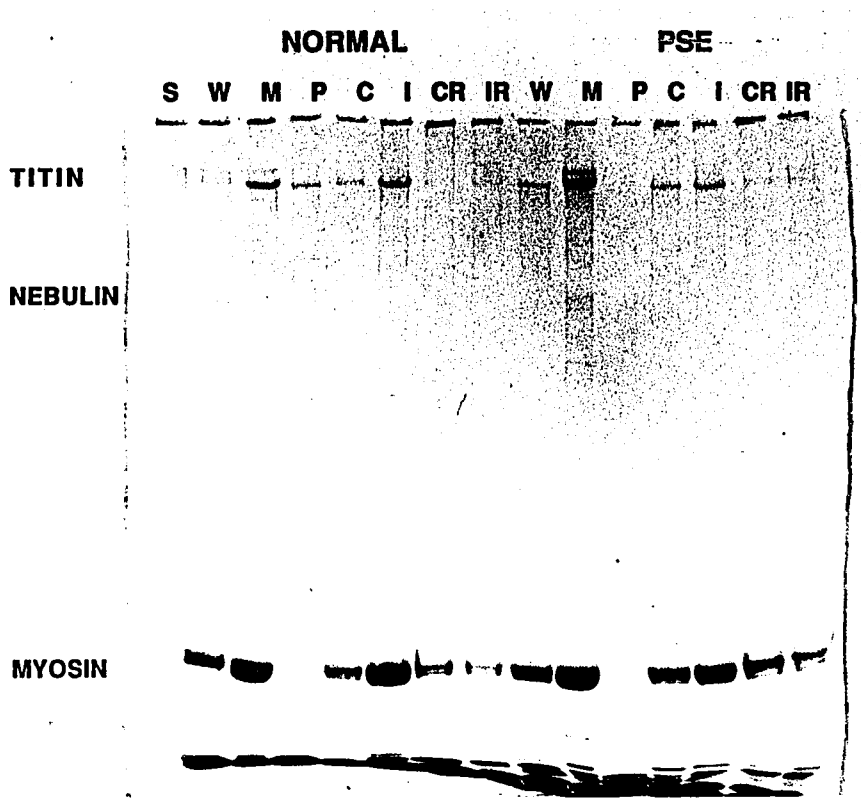

205
116
97.4
66
45
29

Figure 8: Five percent SDS-PAG electrophoretogram of supernatants of 0.03 M $KPO_4$, 0.5 M KCl and 1.1 M KI extractions and residues of 0.5 M KCl and 1.1 M KI extracts, whole muscle homogenates and myofibrils 7 days postmortem from normal and PSE muscle (S= bovine *titin*, W=whole muscle, M=myofibril, P=phosphate extraction, C=potassium chloride extraction, I=potassium iodide extraction, CR=chloride residue, IR=iodide residue) (40 μg of total protein was loaded on each lane)


NORMAL
PSE
S W M P C I CR IR W M P C I CR IR
TITIN
NEBULIN
MYOSIN

observations of Kang and colleagues (1978) and Kolczak and Weber (1969) who reported less protein extracted from PSE muscle, but observed no difference in the number of myofibrillar and sarcoplasmic proteins observed on agarose gels.

Calpain is a protease that is responsible for the calcium dependent degradation of certain myofibrillar proteins postmortem (Olson et al., 1977). Because PSE muscle has an increase in calcium levels in response to stress (Greaser et al., 1969; Topel et al., 1975; Ludvigsen, 1985; Topel and Hallberg, 1985), this suggests that additional calcium could lead to increased postmortem degradation of myofibrillar/cytoskeletal proteins by stimulating calpain activity. The degradation of lower molecular weight proteins, however, was unaffected by the PSE condition (Figure 9). No increase in bands corresponding to lower molecular weight degradation products was observed. Myofibrils isolated from normal muscle, however, had a slight increase in bands around 100 kDa region and below actin starting at day 3 and increasing in intensity at days 5 and 7. These observations agree with Bechtel and Parrish (1983) who saw an increase in a 90 and 110 kDA component on SDS-PAGE in whole muscle samples from bovine *longissimus* muscle stored for 3, 7, and 14 days postmortem. Furthermore, the high molecular weight protein titin was degraded less in PSE muscle samples than those in muscle from normal animals (Figure 10). Upon postmortem aging, $T_1$ is degraded to $T_2$ (Lusby et al., 1983;

Figure 9: Ten percent SDS-PAG electrophoretogram of myofibrils isolated after 0, 1, 3, 5, and 7 days of postmortem storage at 2°C from normal and PSE animals (40 μg of total protein was loaded on each lane) (numbers to left indicate molecular weights in kDa)

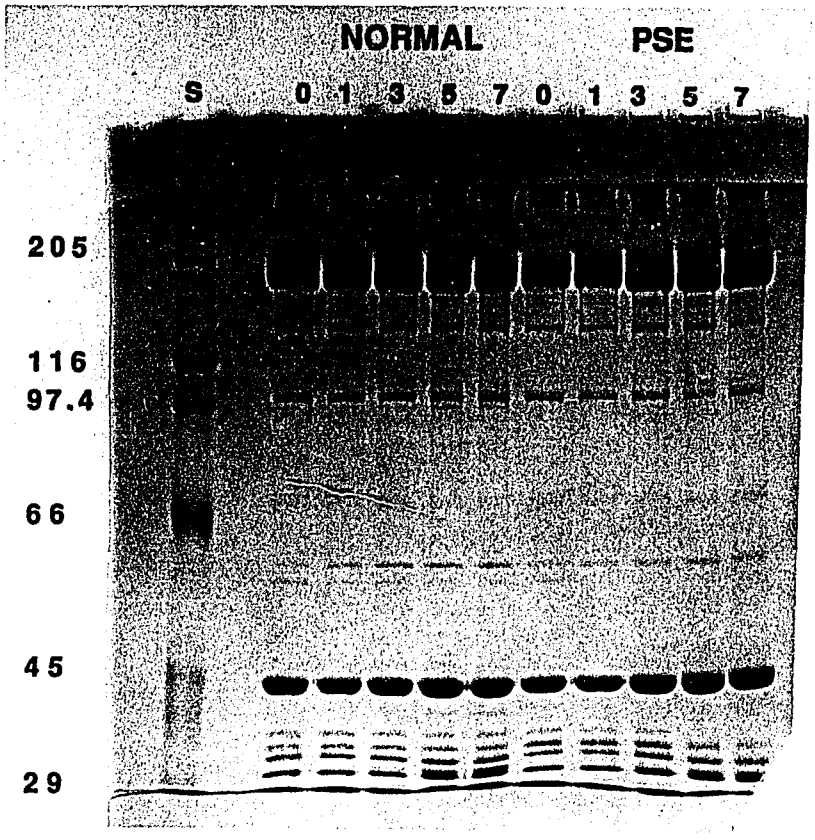
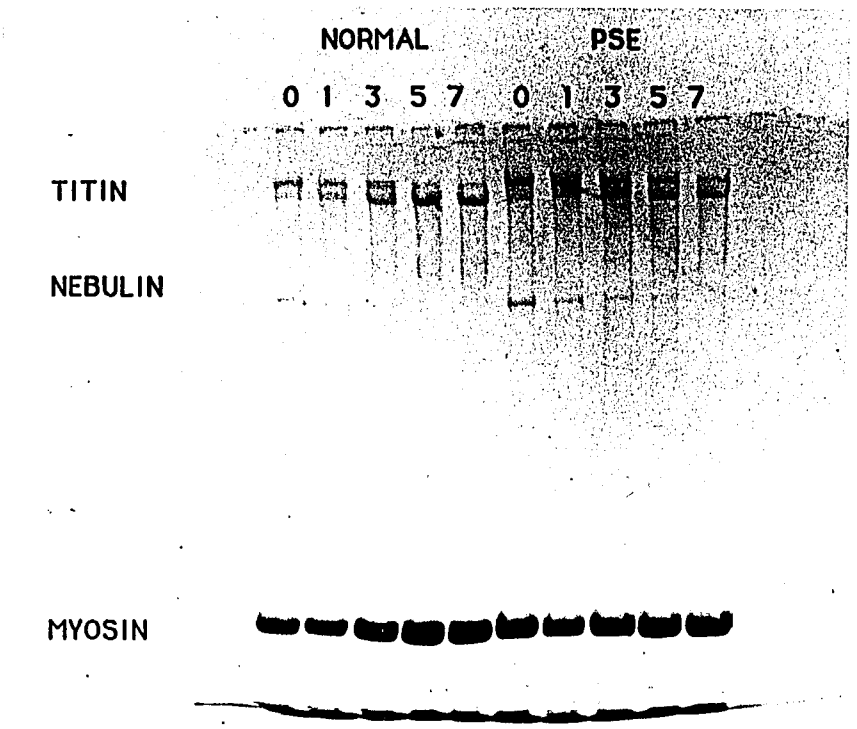

NORMAL
PSE
S
0 1 3 5 7 0 1 3 5 7
205
116
97.4
66
45
29

Figure 10: Five percent SDS-PAG electrophoretogram of myofibrils isolated after 0, 1, 3, 5, and 7 days of postmortem storage at 2°C from normal and PSE animals (40 μg of total protein was loaded on each lane)


NORMAL
PSE
0 1 3 5 7 0 1 3 5 7
TITIN
NEBULIN
MYOSIN

Paterson and Parrish, 1987). This postmortem degradation is coincident with increased tenderness due to aging (Paterson and Parrish, 1986; Anderson and Parrish, 1989). More $T_2$ was observable in myofibrils isolated 5 and 7 days postmortem from normal animals than in the same postmortem time samples from PSE animals. Because titin seems to exist as a doublet in PSE muscle, this may be a reason for reduced water-holding capacity of PSE meat. Offer and Trinick (1983) reported that transverse structural constraints affected the ability of muscle to hold water. They found that the removal of these structural constraints, myosin cross-bridges, Z- and M-lines, resulted in greater water-holding capacity. Paterson and co-workers (1988) later observed increased extraction of titin with salt and phosphate solutions and a concomitant increase in water-holding capacity, suggesting that the removal of titin from gap filaments was of consequence in improving water-holding capacity. The decrease in degradation of titin suggests that proteolysis has been affected detrimentally by the rapid drop in pH at high carcass temperature in the PSE condition. Hence, calpain was likely modified because of rapid pH drop and high temperature interaction, or autolyzed because of the rapid influx of calcium into the cell associated with the porcine stress syndrome condition. Kang and co-workers (1978) also observed reduced proteolysis when purified calpain was added to myofibrils isolated from PSE muscle, *in vitro*. They attributed this reduction in proteolysis to alterations in the

myofibril to make it more resistant to degradation or calpain from PSE muscle has a lower activity or is more labile than that from normal muscle.

The research reported here is the first attempt to determine what happened to individual muscle proteins, especially the myofibrillar/cytoskeletal proteins, titin and nebulin, in the *longissimus* muscle with the development of pale, soft, exudative muscle. In summary, the solubility of sarcoplasmic and myofibrillar/cytoskeletal proteins in *longissimus* muscle is diminished in muscle with the PSE condition. Furthermore, the inhibition of degradation of titin, as evidenced by the presence of $T_1$ 7 days postmortem, may have an adverse affect on the water-holding capacity of the muscle tissue. The combination of lowered protein solubility and reduced degradation of structural constraints (presence of $T_1$ and $T_2$ on SDS-PAGE) may explain the reduced water-holding capacity and functionality of PSE muscle.

Acknowledgements

We gratefully acknowledge the support of the National Livestock and Meat Board. We also thank Kathy Schneider, Maureen Dolan and Susan Joens for their technical support.

## REFERENCES

Allen, W. M., and Patterson, D. S. P. 1971. The possible relationship between plasma creatine phosphokinase activity and muscle characteristics in the pig. Pages 90-96 in J. C. M. Hasses-de Herr, G. R. Schmidt, W. Sybesma, and P. G. van der Wal, eds. Proc. of the 2nd International Symposium on Conditions and Meat Quality of Pigs. Centre of Agriculture Publishing and Documentation, Wageningen, The Netherlands.

Anderson, T. J. and Parrish, Jr., F. C. 1989. Postmortem degradation of titin and nebulin of beef steaks varying in tenderness. J. Food Sci. 54:748.

Bechtel, P. J. and Parrish, Jr., F. C. 1983. Effects of postmortem storage and temperature on muslce protein degradation: Analysis by SDS gel electrophoresis. J. Food Sci. 48:294.

Bendall, J. R. and Wismer-Pedersen, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. J. Food Sci. 27:144.

Chaudry, H. M., Parrish, Jr., F. C. and Goll D. E. 1969. Molecular properties of post-mortem muscle. 6. Effect of temperature on protein solubility of rabbit and bovine muscle. J. Food Sci. 34:183.

Cheah, K. S., Cheah, A. M., Crosland, A. R., Casey, J. C., and Webb, A. J. 1984. Relationship between $Ca^{2+}$ release, sarcoplasmic $Ca^{2+}$, glycolysis and meat quality in halothane-sensitive and halothane-insensitive pigs. Meat Sci. 10:117.

Christian, L. L. 1974. Halothane test for PSS-field application. Pages 6-13 in Proc. Am. Assn. Swine Practitioners Conf., Des Moines, Iowa.

Dayton, W. R., Goll, D. E., Reville, W. R., Zeece, M. G., Stromer, M. H., and Robson, R. M. 1974. Purification and some properties of muscle enzyme that degrades myofibril. Fed. Proc. 33:1580. Abstr.

Dayton, W. R., Reville, W. J., Goll, D. E., and Stromer, M. H. 1976. A $Ca^{2+}$-activated protease possibley involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochemistry 15:2159.

Eikelenboom, G. and van den Bergh, S. G. 1973. Mitochondrial metabolism in stress-susceptible pigs. J. Anim. Sci. 37:692.

Fritz, J. D., Swartz, D. R., and Greaser, M. L. 1989. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. Anal. Biochem. 180:205.

Greaser, M. L., Cassens, R. G., Briskey, E. J., and Hoekstra, W. G. 1969. Post-mortem changes in subcellular fractions from normal and pale, soft, exudative porcine muscle. 1. Calcium accumulation and adenosine triphosphatase activities. J. Food Sci. 34:120.

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.

Jensen, P. and Barton-Gade, P. A. 1985. Performance and carcass characteristics of pigs with known genotypes for halothane susceptibility. EAAP Publication 33:80.

Kang, C. G., Muguruma, M., Fukazawa, T., andIto, T. 1978. Difference in Z-line removal between normal and PSE porcine myofibrils. J. Food Sci. 43:509.

Kolczak, T. and Weber, M. 1969. Water extractable proteins of porcine muscle. J. Food Sci. 34:547.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteroiphage T4. Nature 227:680.

Ludvigsen, J. B. 1985 Intermediary magnesium-and calcium metabolism in stress-susceptible pigs. EAAP Publications 33:106.

Lusby, M. L., Ridpath, J. F., Parrish, Jr., F. C., and Robson, R. M. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine *longissimus* muscle. J. Food Sci. 48:1787.

MacBride, M. A. and Parrish, Jr., F. C. 1977. The 30,000 dalton component of tender bovine longissimus muscle. J. Food Sci. 42:1627.

McLoughlin, J. V. and Goldspink, G. 1963. Exudative pig muscle. Irish J. Agric. Res. 2:27.

Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci. 8:245.

Olson, D. G., Parrish, Jr., F. C., Dayton, W. R. and Goll, D. E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42:117.

Paterson, B. C. and Parrish, Jr., F. C. 1986. A sensory panel and chemical analysis of certain beef chuck muscles. J. Food Sci. 51:876.

Paterson, B. C. and Parrish, Jr., F. C. 1987. SDS-PAGE conditions for detection of titin and nebulin in tender and tough bovine muscles. J. Food Sci. 52:509.

Paterson, B. C., Parrish, F. C., Jr., and Stromer, M. H. 1988. Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle. J. Food Sci. 53:1258.

Penny, I. F. 1977. The effect of temperature on the drip, denaturation and extracellular space of pork *Longissimus dorsi* muscle. J. Sci. Food Agric. 28:329.

Rasmusen, B. A. and Christian, L. L. 1976. H blood types in pigs as predictors of stress-susceptibility. Science 191:947.

Reddy, M. K., Etlinger, J., Zak, R., Fischman, D. A., and Rabinowitz, M. 1974. Specific removal of Z-lines and α-actinin from isolated skeletal muscle myofibrils and by a $Ca^{2+}$activated neutral protease. Fed. Proc. 33:1580. Abstr.

Robson, R. M., Goll, D. E., and Temple M. J. 1968. Determination of protein in "tris" buffer by the biuret reaction. Anal. Biochem. 24:339.

SAS Institute Inc. 1985a. SAS User's Guide: Basics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

SAS Institute Inc. 1985b. SAS User's Guide: Statistics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

Sayre, R. N. and Briskey, E. J. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28:675.

Swatland, H. J. 1986. Color measurements on pork and veal carcasses by fiber optic spectrophotometry. Can. Inst. Food Sci. Technol. J. 19:170.

Szent-Gyorgyi, A. G. 1951. The reversible depolymerization of actin by potassium iodide. Arch. Biochem. Biophys. 31:97.

Takahashi, K. and Saito, H. 1979. Post-mortem changes in skeletal muscle connectin. J. Biochem. 85:1539.

Topel, D. G. and Hallberg, J. W. 1985. Stress-susceptibility with particular emphasis on carcass quality and health. EAAP Publication 33:49.

Topel, D. G., Miller, J. A., Berger, P. J., Rust, R. E., Parish, Jr., F. C., and Ono, K. 1976. Palatability and visual acceptance of dark, normal and pale colored *M. longissimus*. J. Food Sci. 41:628.

Topel, D. G., Staun, H., and Riis, H. M. 1975. Relationships between stress adaptation traits in swine with skeletal muscle characteristics. World Rev. Anim. Prod. 10:52.

Valin, C. 1968. Post-mortem changes in myofibrillar protein solubility. J. Food Technol. 3:171.

Wang, K. 1982. Purificaiton of titin and nebulin. Methods in Enzymol. 85:264.

Wang, K., McClure, J., and Tu, A. 1979. Titin: Major myofibrillar components of striated muscle. Proc. Natl. Acad. Sci. 76:3698.

Warriss, P. D. 1982. The relationship between $pH_{45}$ and drip in pig muscle. J. Food Technol. 17:573.

## SUMMARY

Researchers have suggested that exogenous growth hormone treatment increases the incidence of pale, soft exudative, meat (Solomon et al., 1989; Solomon et al., 1990). Pale, soft, exudative muscle is usually associated with stress susceptible animals (Topel et al., 1975; Cheah et al., 1984). As a result of stress, PSE muscle displays a rapid postmortem pH decline (Eikelenboom and van den Bergh, 1973; Warriss, 1982), a reduction in water-holding capacity (Penney, 1977; Warriss, 1982) and decreased protein solubility (Bendall and Wismer-Pedersen, 1962; Sayre and Briskey, 1963). Consequently, this condition leads to a loss of functionality of the proteins in processed products and large weight losses in fresh and processed products. Because of these problems along with the decreased fat, processors are concerned about the effect of porcine somatotropin on the processing and palatability characteristics of the muscle from these animals. The objective of this study was to determine the effects of exogenous porcine somatotropin on the sensory, chemical and physical properties of skeletal muscle from three stress classification of pigs. Also the effects of stress susceptibility on the solubility and degradation of the sarcoplasmic and myofibrillar/cytoskeletal proteins were investigated.

Pigs of three known genotypes, stress positive, stress carrier and stress negative, were injected daily with recombinant porcine somatotropin (PST) (4 mg/day) or placebo. The effects of PST and stress on the sensory, physical and processing characteristics of pork skeletal muscle were observed. Loins and

*semimembranosus* (SM) muscles were obtained 24 hr postmortem, vacuum-packaged and frozen until analysis (-29°C). Chops (1.91 cm) were cut from frozen primal loins and thawed at 2°C. For sensory analysis, chops were cooked to two end-point temperatures (71° and 77°C). Sensory analysis of loin chops was done by a 13-member panel using a 15-cm line scale. SM muscles were thawed, pumped to 25% of their original weight and processed into hams. Sensory analysis of hams was done by an inexperienced panel consisting of approximately 30 Iowa State University faculty, staff and students using a 7-point facial hedonic scale. Color, proximate composition, and percent cook yields were done for both loin chops and hams, and water-holding capacity was determined for the raw loin chops.

Porcine somatotropin can be used successfully to lower tissue lipid without major detrimental effects upon sensory and processing characteristics. PST treatment of animals did significantly ($P<0.05$) lower sensory scores for tenderness, juiciness and flavor, but the changes were small. Furthermore, PST treatment of animals had no effect on the sensory or processing characteristics of hams, and treatment of animals with PST did not increase the incidence of pale, soft exudative muscle, as identified by 45-min pH and color measures. These results confirm those reported by others who reported a non-significant decrease (Novakofski et al., 1988; Beerman et al., 1988a; Prusa et al., 1990) in palatability of pork chops from PST treated animals.

However, it disagrees with data (Solomon et al., 1989; Solomon et al., 1990) that showed that PST treatment increases the incidence of pale, soft, exudative pork.

Stress susceptibility, however, significantly ($P<0.05$) lowered sensory scores for tenderness of loin chops. In addition, sensory scores of hams from PSE muscle were significantly ($P<0.05$) lower for texture, flavor and overall palatability. Processing yields were also significantly ($P<0.05$) lowered when PSE muscle was used. These results confirm observations of other researchers who observed reduced acceptability and processing yields of hams when made from PSE muscle (Wismer-Pedersen, 1960; Merkel, 1971).

Cooking to higher end-point temperatures significantly ($P<0.05$) decreased sensory panel scores for tenderness and juiciness, but did not effect flavor scores. Cooking to higher end-point temperature, also significantly ($P<0.05$) reduced cook yield. These results indicates that cooking to an end-point of 71°C increases the palatability of pork chops as well as product yields.

The solubility of sarcoplasmic and myofibrillar/cytoskeletal proteins in *longissimus dorsi* muscle is diminished in muscle with the PSE condition. Furthermore, the inhibition of degradation of titin, as evidenced by the presence of $T_1$ 7 days postmortem, may have an adverse affect on the water-holding capacity of the muscle tissue. The combination of lowered protein solubility and reduced degradation of structural constraints (presence of $T_1$ and

$T_2$ on SDS-PAGE) may in part explain the reduced water-holding capacity and functionality of PSE muscle.

## CONCLUSIONS

The following conclusions are derived from this study:

1. Treatment of animals with PST does not increase the incidence of pale, soft, exudative loin chops and hams.
2. Loin chops from the PST treatment have lower sensory scores for tenderness, juiciness and flavor.
3. PST treatment of pigs decreases fat content and increases moisture content of *longissimus* muscle.
4. Loin chops from animals treated with PST have significantly lower Hunter L values than chops from untreated animals, indicating a darker color.
5. Loin chops from stress susceptible animals are significantly less tender than chops from normal animals.
6. Loin chops from stress positive animals have a lean color with greater Hunter L vales, indicating a lighter color.
7. Cooking to a lower end-point temperature (71°C) significantly improves tenderness and juiciness of broiled pork loin chops.
8. PST treatment has no effect on the processing or palatability characteristics of hams, especially cured lean color.
9. Hams from stress susceptible animals have lower processing yields and palatability scores.
10. Protein solubility is reduced in *longissimus* muscle from stress susceptible animals.
11. Protein solubility in *longissimus* muscle was not affected by postmortem storage.

12. Titin is not degraded in myofibrils isolated from stress susceptible animals based on results obtained by using SDS-PAGE.
13. The combination of reduced protein solubility and titin degradation may explain the reduced fuctionality of muscle from stess susceptible animals.
14. Muscle from animals identified as stress carriers was not different from normal animals in any of the measures.

## REFERENCES

A.O.A.C. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D. C.

Abbott, J. A. 1973. Sensory assessment of textural attributes of foods. p. 17. In A. Kramer and A. S. Szczesniak, ed. Texture Measurements of Foods. D. Reidel Publishing Company, Dordecht, Holland.

Allen, R. E. 1988. Muscle cell growth and development. p. 142. In Designing Foods. National Academy of Sciences,Washington, D. C.

Allen, R. E., Merkel, R. A., and Young, R. B. 1979. Cellular aspects of muscle growth: Myogenic cell proliferation. J. Anim. Sci. 49:115.

Allen, R. E., Young, R. G., Stromer, M. H., and Goll, D. E. 1975. Myogenesis. Proc. Recip. Meat Conf. 28:182.

Allen, W. M., and Patterson, D. S. P. 1971. The possible relationship between plasma creatine phosphokinase activity and muscle characteristics in the pig. Pages 90-96 in J. C. M. Hasses-de Herr, G. R. Schmidt, W. Sybesma, and P. G. van der Wal, eds. Proc. of the 2nd International Symposium on Conditions and Meat Quality of Pigs. Centre of Agriculture Publishing and Documentation, Wageningen, The Netherlands.

Andersen, L. D., Parrish, Jr., F. C., and Topel, D. G. 1975. Histochemical nad palatability properties of *M. longissimus* from stress-resistant and stress-susceptible porcine animals. J. Anim. Sci. 41:1600.

Anderson, T. J. and Parrish, Jr., F. C. Postmortem degradation of titin and nebulin of beef steaks varying in tenderness. J. Food Sci. 54:748.

Baile, C. A., Della-Fera, M. A., and McLaughlin, C. L. 1983. Performance and carcass quality of swine injected daily with bacterially-synthesized human growth hormone. Growth 47:225.

Bark, L. J., Stahly, T. S., and Cromwell, G. L. 1989. Influence of genetic capacity for lean tissue growth on responses of pigs to recombinant porcine somatotropin. J. Anim. Sci. 67(Suppl. 1): 212. Abstr.

Barton-Gade, P. A. 1988. The effects of breed on meat quality characteristics in pigs. Proc. Internat'l. Congress Meat Sci. Technol. 34:568.

Bechtel, P. J. and Parrish, Jr., F. C. 1983. Effects of postmortem storage and temperature on muslce protein degradation: Analysis by SDS gel electrophoresis. J. Food Sci. 48:294.

Bechtel, P. J., Easter, R. A., McKeith, F. K., Novakofski, J., McLaren, D. G., and Grebner, G. L. 1988. Growth, carcass and sensory characteristics for pigs injected daily with natural porcine somatotropin. Proc. Internat'l. Congress Meat Sci. Technol. 34:603.

Beerman, D. H., Ambruster, G., Boyd, R. D., Roneker, K. and Fagin, K. D. 1988a. Comparision of the effects of two recombinant forms of porcine somatotropin (pST) on pork composition and palatability. J. Anim. Sci. 66 (Suppl. 1):281.

Beerman, D. H., Boyd, R. D., Armbruster, G., DeNeergard, A. F., Roneker, K., Bartley, T. D., and Fagin, K. D. 1988b. Dose response effects of native and recombinant porcine somatotropin (pST) on growth performance, composition of gain and pork quality. Proc. Internat'l. Congress Meat Technol. 34:601.

Bendall, J. R. and Wismer-Pedersen, J. 1962. Some properties of the fibrillar proteins of normal and watery pork muscle. J. Food Sci. 27:144.

Bouton, P. E., Harris, P. V., and Shorthose, W. R. 1972. The effects of ultimate pH on ovine muscle: Water-holding capacity. J. Food Sci. 37:351.

Chaudry, H. M., Parrish, Jr., F. C. and Goll D. E. 1969. Molecular properties of post-mortem muscle. 6. Effect of temperature on protein solubility of rabbit and bovine muscle. J. Food Sci. 34:183.

Cheah, K. S. 1973. Comparative studies of the mitochondrial properties of *longissimus dorsi* muscles of Pietrain and Large White pigs. J. Sci. Food Agric. 24:51.

Cheah, K. S., Cheah, A. M., Crosland, A. R., Casey, J. C., and Webb, A. J. 1984. Relationship between $Ca^{2+}$ release, sarcoplasmic $Ca^{2+}$, glycolysis and meat quality in halothane-sensitive and halothane-insensitive pigs. Meat Sci. 10:117.

Christian, L. L. 1974. Halothane test for PSS-field application. Pages 6-13 in Proc. Am. Assn. Swine Practitioners Conf., Des Moines, Iowa.

Chung, C. S., Etherton; T. D., and Wiggins, J. P. 1985. Stimulation of swine growth by porcine growth hormone. J. Anim. Sci. 60:118

Craig, R., and Offer, G. 1976.The isolation of C-protein in rabbit skeletal muscle. Proc. R. Soc., London, B., 192:451.

Davey, C. L. and Winger, R. J. 1980. The structure of skeletal muscle and meat toughness. p. 97. In D. A. D. Perry and L. K. Creamer, ed. Fibrous Proteins: Scientific, Industrial and Medical Aspects. Volume I. Academic Press, New York, New York.

Dayton, W. R. 1988. The use of bioassays to detect and isolate protein or peptide factors regulating muscle growth in meat-producing animals. p. 173. In Designing Foods. National Academy Press, Washington, D. C.

Dayton, W. R., Goll, D. E., Reville, W. R., Zeece, M. G., Stromer, M. H., and Robson, R. M. 1974. Purification and some properties of muscle enzyme that degrades myofibril. Fed. Proc. 33:1580. Abstr.

Dayton, W. R., Reville, W. J., Goll, D. E., and Stromer, M. H. 1976. A $Ca^{2+}$-activated protease possibly involved in myofibrillar protein turnover. Partial characterization of the purified enzyme. Biochemistry 15:2159.

dos Remedios, C. G. and Gilmour, D. 1978. Is there a third type of filament in striated muscles? J. Biochem. 84:235.

Dunshea, F. R., Harris, D. M., Bauman, D. E., Boyd, R. D., and Bell, A. W. 1989. Effect of porcine somatotropin (pST) on glucose metabolism and lipid synthesis in the growing pig: an *in vivo* approach. J. Anim. Sci. 67(Suppl. 1):216. Abstr.

Eikelenboom, G. 1985. Ways to improve meat quality in pigs. EAAP Publication 33:68.

Eikelenboom, G. and Nanni Costa, L. 1988. Fibre optic probe measurements in Landrace pigs of different halothane phenotypes. Proc. Internat'l. Congress Meat Sci. Technol. 34:578.

Eikelenboom, G. and van den Bergh, S. G. 1973. Mitochondrial metabolism in stress-susceptible pigs. J. Anim. Sci. 37:692.

Eisemann, J. H., Hammond, A. C., Bauman, D. E., Reynolds, P. J., McCutcheon, S. N., Tyrrell, H. F., and Haaland, G. L. 1986. Effect of bovine growth hormone administration on metabolism of growing Hereford heifers: Protein and lipid metabolism and plasma concentrations of metabolites and hormones. J. of Nutr. 116:2504.

Ender, K., Lieberenz, M., Poppe, S., Hackl, W., Pflughaupt, G., and Meisinger, D. 1989a. Effect of porcine somatotropin (pST) treatment on growing-finishing pigs: Carcass characteristics. J. Anim. Sci. 67(Suppl. 1):212. Abstr.

Ender, K., Lieberenz, M., Poppe, S., Hackl, W., Pflughaupt, G., and Meisinger, D. 1989b. Effect of porcine somatotropin (pST) treatment on growing-finishing pigs: Performance. J. Anim. Sci. 67(Suppl. 1):211. Abstr.

Erlander, M G., Parliman, J. A., Draper, D. D., Christian, L. L., Murrin, L. C., Pfeiffer, R. F., and Beitz, D. C. 1985. Effects of L-DOPA supplementation on concentrations of brain catechols, dopamine receptor binding and the incidence of pale, soft and exudative meat in stress-susceptible pigs. J. Anim. Sci. 61:914.

Essen-Gustavsson, B., Karlstrom, K., and Lundstrom, K. 1988. Muscle metabolism and glycogen depletion within fibre types at slaughter in pigs with different halothane genotypes. Relation to meat quality properties. Proc. Internat'l. Congress Meat Sci. Technol. 34:581.

Etherton, T. D., Wiggins, J. P., Chung, C. S., Evock, C. M., Rebhun, J. F., and Walton, P. E. 1986. Stimulation of pig growth performance by porcine growth hormone and growth hormone releasing factor. J. Anim. Sci. 63:1389.

Etherton, T. D., Wiggins, J. P., Evock, C. M., Chung, C. S., Rebhun, J. F., Walton, P. E., and Steele, N. C. 1987. Stimulation of pig growth performance by porcine growth hormone: Determination of the dose-response relationship. J. Anim. Sci. 64:433.

Ewan, R. C., Topel, D. G., and Ono, K. 1979. Chemical composition of chops from pale, soft, exudative (PSE) and normal pork loins. J. Food Sci. 44:678.

Fjelkner-Modig, S. 1986. Sensory properties of pork, as influenced by cooking temperature and breed. J. Food Qual. 9:89.

Forrest, J. C., Aberle, E. D., Hedrick, H. B., Judge, M. D., and Merkel, R. A. 1975. Structure and composition of muscle and associated tissues. In B. S. Schweigert, ed. Principles of Meat Science. W. H. Freeman and Company, San Francisco.

Fritz, J. D., Swartz, D. R., and Greaser, M. L. 1989. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. Anal. Biochem. 180:205.

Furst, D. O., Osborn, M., Nave, R., and Weber, K. 1988. The organization of titin filaments in the half-sarcomere revealed by monoclonal antibodies in immunoelectron microscopy: A map of ten nonrepetitive epitopes starting at the Z-line extends close to the M-line. J. Cell Biol. 106:1563.

Goll, D. E., Edmunds, T., Kleese, W. C., Sathe, S. K., and Shannon, J. D. 1985. Some properties of the $Ca^{2+}$-dependent proteinase. p. 151. In I. A. Khairallah, J. S. Bond, and J. W. C. Bird eds. Intracellular Protein Catabolism. Alan R. Liss, Inc., New York, NY.

Goll, D. E., Robson, R. M., and Stromer, M. H. 1984. Skeletal Muscle. p. 548. In M. J. Swenson, ed. Duke's Physiology of Domestic Animals. 10th Edition. Cornell University Press, Ithaca, New York.

Greaser, M. L., Cassens, R. G., Briskey, E. J., and Hoekstra, W. G. 1969a. Post-mortem changes in subcellular fractions from normal and pale, soft. exudative porcine muscle. 1. Calcium accumulation and adenosine triphosphatase activities. J. Food Sci. 34:120.

Grove, B. K. and Eppenberger, H. M. 1983. Appearance of myomesin and M-protein during chicken muscle development. J. Cell Biol. 97:551a. Abstr.

Hainfeld, J. F., Wall, J. S., and Wang, K. 1988. Titin: quantitative mass measurements by scanning transmission electron microscopy and structural implications for the sarcomere matrix of skeletal muscle. FEB 234:145.

Hamm, R. 1960. Biochemistry of meat hydration. Adv. Food Res. 10:355.

Hart, P. C. 1962. Physico-chemical characteristics of degenerated meat in pigs II. Tijdscrift voor Diergeneskunde 87:156. Abstr.

Heald, R. W. and Hitchcock-DeGregoris, S. E. 1988. The structure of the amino terminus of tropomyosin is critical for binding to actin in the absence and presence of troponin. J. Biol. Chem. 263(11):5254.

Heymann, H., Hedrick, H. B., Karrasch, M. A., Eggeman, M. K., and Ellersieck, M. R. 1990. Sensory and chemical characteristics of fresh pork roasts cooked to different endpoint temperatures. J. Food Sci. 55:613.

Honikel, K. A. and Fischer, C. 1977. A rapid method for the detection of PSE and DFD pork muscles. J. Food Sci. 42:1633.

Honikel, K. O. and Fischer, C. 1980. Rapid method for the detection of PSE and DFD meat. Proc Eur. Meeting Meat Res. Workers 26:6.

Honikel, K. O., Fischer, C., Hamid, A., and Hamm, R. 1981a. Influence of postmortem changes in bovine muscle on the water-holding capacity of beef. Postmortem storage of muscle at 20°C. J. Food Sci. 46:1

Honikel, K. O., Hamid, A., Fischer, C., and Hamm, R. 1981b. Influence of postmortem changes in water-holding capacity of beef. Postmortem storage of muscle at various temperatures between 0° and 30° C. J. Food Sci. 46:23.

Horowits, R. and Podolsky, R. J. 1987. The positional stability of thick filaments in activated skeletal muscle depends on sarcomere length: Evidence for the role of titin filaments. J. Cell Biol. 105:2217.

Horowits, R., Kempner, E. S., Bisher, M. E., and Podolsky, R. J. 1986. A physiological role for titin and nebulin in skeletal muscle. Nature 323:160.

Horowits, R., Maruyama, K., and Podolsky, R. J. 1989. Elastic behavior of connectin filaments during thick filament movement in activated skeletal muscle. J. Cell Biol. 109:2169.

Huiatt, T. W., Robson, R. M., Arakawa, N., and Stromer, M. H. 1980. Desmin from avian smooth muscle: Purification and partial characterization. J. Biol. Chem. 255:6981.

Huxley, H. E. 1958. The contraction of muscle. Scientific American 206(6):19.

Huxley, H. E. 1965. The mechanism of muscular contraction. Scientific American 213(6):18.

Ishira, S., Sugita, H., Nonaka, I., and Imahori, K. 1980. Calcium-activated neutral protease. Its localization in the myofibril, especially at the Z-band. J. Biochem. 87:343.

Jensen, P. and Barton-Gade, P. A. 1985. Performance and carcass characteristics of pigs with known genotypes for halothane susceptibility. EAAP Publication 33:80.

Jones, S. D. M., Fortin, A., and Atin, M. 1984. A comparison of methods to detect pork quality 24 hours post mortem from measurements made within one hour of slaughter. Can Inst. Food Sci. Technol. J. 17:143.

Jorgensen, P. F. 1985. Some aspects of electrolyte metabolism related to malignant hyperthermia and stress susceptibility in pigs. EAAP Publication 33:88.

Kang, C. G., Muguruma, M., Fukazawa, T., and Ito, T. 1978. Difference in Z-line removal between normal and PSE porcine myofibrils. J. Food Sci. 43:509.

Kanis, E., Vander Hel, W., Kouwenberg, W. J. A., Huisman, J., Politiek, R. D., Verstegen, M. W. A., Van der Wal, P.. And Van Weerden, E. J. 1988. Effects of recombinant porcine somatotropin (rPST) on meat quality of pigs. J. Anim. Sci. 66 (Suppl. 1):280.

Kauffman, R. G., Eikelenboom, G., Van der Wal, P. G., Engel, B., and Zaar, M. 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18:307.

Kimura, S. and Maruyama, K. 1983. Preparation of native connectin from chicken breast muscle. J. Biochem. 94:2083.

King, N. L. and Kurth, L. 1980. SDS gel electrophoresis studies of connectin. In D. A. Perry and L. K. Creamer, eds. Fibrous Proteins: Scientific, Industrial and Medical Aspects. Volume 2. Academic Press, London.

Kishimoto, A., Kajikawa, N. Tabuchi, H., Shiota, M., and Nishizuka, Y. 1981. Calcium-dependent neutral proteae active at lower concentrations of calcium. J. Biochem. 90:889.

Kolczak, T. and Weber, M. 1969. Water extractable proteins of porcine muscle. J. Food Sci. 34:547.

Koohmaraie, M., Babiker, A. S., Merkel, R. A., and Dutson, T. R. 1988b. Role of $Ca^{++}$-dependent proteases and lysosomal enzymes in postmortem changes in bovine skeletal muscle. J. Food Sci. 53:1253.

Koohmaraie, M., Babiker, A. S., Schroeder, A. L., Merkel, R. A., and Dutson, T. R. 1988c. Acceleration of postmortem tenderization in ovine carcasses through activation of $Ca^{2+}$-dependent proteases. J. Food Sci. 53:1638.

Koohmaraie, M., Seideman, S. C., Schollmeyer, J. E., Dutson, T. R., and Babiker, A. S. J. 1988a. Factors associated with the tenderness of three bovine muscles. J. Food Sci. 53:407.

Kotts, C. E., Buonomo, F., White, M. E., Allen, C. E., and Dayton, W. R. 1987. Stimulation of *in vitro* muscle cell proliferation by sera from swine injected with porcine growth hormone. J. Anim. Sci. 64:623.

Kramer, A. 1973. Food texture: Definition, measurement and relation to other food quality attributes. p. 1. In A. Kramer and A. S. Szczesniak, ed. Texture Measurements of Foods. D. Reidel Publishing Company, Dordecht, Holland.

Kurzban, G. P. and Wang, K. 1988. Giant polypeptides of skeletal muscle titin: Sedimentation equilibrium in guanidine hydrochloride. Biochem. Biophys. Res. Commun. 150:1155.

Laakkonen, E. 1973. Factors affecting tenderness during heating of meat. Adv. Food Res. 20:257.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteroiphage T4. Nature 227:680.

Locker, R. H. and Leet, N. G. 1975. Histology of highly-stretched beef muscle I. The fine structure of grossly stretched single fibers. J. Ultrastruct. Res. 52:64.

Locker, R. H., Daines, G. J., Carse W. A., and Leet, N. G. 1977. Meat tenderness and the gap filaments. Meat Sci. 1:87.

Ludvigsen, J. B. 1985 Intermediary magnesium and calcium metabolism in stress-susceptible pigs. EAAP Publications 33:106.

Lundstrom, K., Barton-Gade, P., Rud-Andersen, J., and Hansson, I. 1988. Pale pig meat-relative influence of PSE and low pigment content. Proc. Internat'l Congress Meat Sci. Technol. 34:584.

Lusby, M. L., Ridpath, J. F., Parrish, Jr., F. C., and Robson, R. M. 1983. Effect of postmortem storage on degradation of the myofibrillar protein titin in bovine *longissimus* muscle. J. Food Sci. 48:1787.

MacBride, M. A. and Parrish, Jr., F. C. 1977. The 30,000 dalton component of tender bovine longissimus muscle. J. Food Sci. 42:1627.

Mak, A. S., Smillie, L. B., and Stewart, G. R. 1980. A comparison of the amino acid sequences of rabbit skeletal muscle a and b tropomyosin. J. Biol. Chem. 255(8):3647.

Marple, D. N. 1977. Physiological factors causing stress-susceptibility. Proc. Recip. Meat Conf. 30:170.

Martin, R. J. and Ezekwe, M. O. 1975. Postnatal muscle growth. Proc. Recip. Meat Conf. 28:48.

Maruyama, K., Kimura, S., Toyota, N. and Ohashi, K. 1980 Connectin, an elastic protein of muscle. In D. A.. Perry and K. L. Creamer, ed. Fibrous Proteins: Scientific, Industrial and Medical Aspects. Academic Press, London.

McClain, P. E., Pearson, A. M., Brunner, J. R., and Crevasse, G. A. 1969. Connective tissues from normal and PSE porcine muscle. 1. Chemical characterization. J. Food Sci. 34:115.

McLoughlin, J. V. and Goldspink, G. 1963a. Exudative pig muscle. Irish J. Agric. Res. 2:27.

McLoughlin, J. V. and Goldspink, G. 1963b. Post-mortem changes in the colour of pig *longissimus dorsi* muscle. Nature 198:584.

Meilgaard, M., Civille, G. V., and Carr, B. T. 1987a. Sensory Evaluation Techniques. Volume I. CRC Press, Inc., Boca Raton, FL.

Meilgaard, M., Civille, G. V., and Carr, B. T. 1987a. Sensory Evaluation Techniques. Volume II. CRC Press, Inc., Boca Raton, FL.

Merkel, R. A. 1971. Processing and organoleptic properties of normal and PSE porcine muscle. Proc. Intl. Symp. Condition Meat Qual. 2:261.

Miller, M. F., Young, L. L., George, S. M., Lyons C. E., Kasser, T. R., and Reagan, J. O. 1989. Effect of calcium and state of rigor on the binding and textural properties of hams from somatotropin (PST) treated pigs. J. Anim. Sci. 67(Suppl. 1):152. Abstr.

Moos, C. 1981. Fluorescence microscope study of the binding of added C-protein to skeletal muscle myofibrils. J. Cell Biol. 90:25.

Murachi, T. 1990. Intracellular regulatory system involving calpain and calpastatin. Biochem. Internat'l. 18:263.

Murayama, T., Nakauchi, Y., Kimura, S., and Maruyama, K. 1989. Binding of connectin to myosin filaments. J. Biochem. 105:323.

Novakofski, J. E., McKeith, F. K., Grebner, G. L., McLaren, D. G., Brenner, M. K., Easter, R. A., Bechtel, P. J., Jones, R. W. and Ingle, D. L. 1988. Pig carcass and pork sensory characteristics for animals given daily injections of natural or recombinant porcine somatotropin from 57 to 108 kg. British Society of Animal Production Winter Meeting, Midlothian, Scotland.

O'Shea, J. M., Robson, R. M., Hartzer, M. K., Huiatt, T. W., Rathbun, W. E., and Stromer, M. H. 1981. Purification of desmin from adult mammalian skeletal muscle. Biochem. J. 195:345.

Ockerman, H. W. and Cahill, V. R. 1968. Water extractability of muscle protein and factors which affect this procedure as a method of determining pork quality. J. Anim. Sci. 27:31.

Offer, G. and Trinick, J. 1983. On the mechanism of water holding in meat: The swelling and shrinking of myofibrils. Meat Sci. 8:245.

Olson, D. G. and Parrish, Jr., F. C. 1977. Relationship of myofibril fragmentation index to measures of beefsteak tenderness. J. Food Sci. 42:506.

Olson, D. G., Parrish, Jr., F. C., Dayton, W. R. and Goll, D. E. 1977. Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci. 42:117.

Parrish, Jr., F.C., Olson, D. G., Miner, B. E., and Rust, R. E. 1973. Effect of degree of marbling and internal temperature of doneness on beef rib steaks. J. Anim. Sci. 37:430.

Paterson, B. C. and Parrish, Jr., F. C. 1986. A sensory panel and chemical analysis of certain beef chuck muscles. J. Food Sci. 51:876.

Paterson, B. C. and Parrish, Jr., F. C. 1987. SDS-PAGE conditions for detection of titin and nebulin in tender and tough bovine muscles. J. Food Sci. 52:509.

Paterson, B. C., Parrish, F. C., Jr., and Stromer, M. H. 1988. Effects of salt and pyrophosphate on the physical and chemical properties of beef muscle. J. Food Sci. 53:1258.

Paxhia, J. M. and Parrish, Jr., F. C. 1988. Effect of postmortem storage on titin and nebulin in pork and poultry light and dark muscles. J. Food Sci. 53:1599.

Penny, I. F. 1969. Protein denaturation and water-holding capacity in pork muscle. J. Food Technol. 4:269.

Penny, I. F. 1977. The effect of temperature on the drip, denaturation and extracellular space of pork *Longissimus dorsi* muscle. J. Sci. Food. Agric. 28:329.

Peters, J. P. 1986. Consequences of accelerated gain and growth hormone administration for lipid metabolism in growing beef steers. J. of Nutr. 116:2490.

Pollard, T. D. and Cooper, J. A. 1986. Actin and actin-binding proteins. A critical evaluation of mechanisms and functions. Ann. Rev. Biochem. 55:987.

Prusa, K. J., Love, J. A., and Miller, L. F. 1989a. Composition and sensory analysis of rib chops from pigs supplemented with porcine somatotropin (pSt). J. Food Qual. 12:455.

Prusa, K. J., Love, J. A., and Miller, L. F. 1989b. Composition, water-holding capacity and pH of muscles from pigs supplemented with porcine somatotropin (pST). J. Food Qual. 12:467.

Prusa, K. J., Sebranek, J. G., Love, J. A., and Miller, L. F. 1990. Quality characteristics of Canadian-style bacon, boneless hams and bacon processed from pigs supplemented with porcine somatotropin. J. Food Sci. In Press.

Rasmusen, B. A. and Christian, L. L. 1976. H blood types in pigs as predictors of stress-susceptibility. Science 191:947.

Reaume, J. B. 1975. Methodology for sensory testing with emphasis on limitations of tests. Proc. Recip. Meat Conf. 28:375.

Reddy, M. K., Etlinger, J., Zak, R., Fischman, D. A., and Rabinowitz, M. 1974. Specific removal of Z-lines and α-actinin from isolated skeletal muscle myofibrils and by a $Ca^{2+}$activated neutral protease. Fed. Proc. 33:1580. Abstr.

Ringkob, T. P., Marsh, B. B., and Greaser, M. L 1988. Change in titin position in postmortem bovine muscle. J. Food Sci. 53:276.

Robson, R. M., Goll, D. E., and Temple M. J. 1968. Determination of protein in "tris" buffer by the biuret reaction. Anal. Biochem. 24:339.

Robson, R. M., Yamaguchi, M., Huiatt, T. W., Richardson, F. L., O'Shea, J. M., Hartzer, M. K., Rathbun, W. E., Schreiner, P. J., Kasang, L. E., Stromer, M. H., Pang, Y.-Y. S., Evans, R. R., Ridpath, J. F. 1981. Biochemistry and molecular architecture of muscle cell 10-nm filaments and Z-line: Roles of desmin and α-actinin. Proc. Recip. Meat Conf. 34:5.

Ruusunen, M. and Puolanne, E. 1988. Fiber-type distribution in porcine muscles. Proc Interna'l Congress Meat Sci. Technol. 34:52.

Sair, R. A., Kastenschmidt, L. L., Cassens, R. G., and Briskey, E. J. 1972. Metabolism and histochemistry of skeletal muscle from stress-susceptibile pigs. J. Food Sci. 37:659.

SAS Institute Inc. 1985a. SAS User's Guide: Basics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

SAS Institute Inc. 1985b. SAS User's Guide: Statistics 1985 Edition. Alice Allen Ray (Ed.). SAS Institute, Inc., Cary, NC.

Sayre, R. N. and Briskey, E. J. 1963. Protein solubility as influenced by physiological conditions in the muscle. J. Food Sci. 28:675.

Sayre, R. N., Kiernat, B., and Briskey, E. J. 1964. Processing characteristics of porcine muscle related to pH and temperature during rigor mortis development and to gross morphology 24 hr. post-mortem. J. Food Sci. 29:175.

Scanes, C. G. and Lauterio, T. J. 1984. Growth hormone: Its physiology and control. J. Exp. Zool. 232:443.

Schwagele, F. and Honikel, K. O. 1988. Studies in post-mortem metabolism of PSE-prone pork muscle. Proc. Internat'l. Congress Meat Sci. Technol. 34:575.

Scopes, R. K. and Lawrie, R. A. 1963. Post-mortem lability of skeletal muscle proteins. Nature 197:1202.

Severini, M., Vizzani, A. and Cenci, G. 1984. The relationship between water-holding capacity, pH and glycolytic rate in swine muscle post mortem. Proc. Eur. Meeting of Meat Res. Workers 30:158.

Shannon, J. D., and Goll D. E. 1985. Properties of a protein that is purified from bovine skeletal muscle that inhibits the $Ca^{2+}$-dependent proteinase. p. 257. In E. A. Kairallah, J. W. C. Bird, and J. S. Bond. eds. Intracellular Protein Catabolism. Alan R. Liss, Inc., New York, NY.

Sjostrand, F. S. 1962. The connections between A- and I-band filaments in striated frog muscle. J. Ultrastruct. Res. 7:225.

Smith, V. G., Kasson, C. W., and Paulissen, J. B. 1989. Growth, feed efficiency and carcass quality of pigs during and after withdrawal of recombinant porcine somatotropin (rpSt). J. Anim. Sci. 67(Suppl. 1):212. Abstr.

Solomon, M. B., Campbell, R. G., and Steele, N. C. 1990. Effect of sex and exogenous porcine somatotropin on *longissimus* muscle fiber characteristics of growing pigs. J. Anim. Sci.68:1176.

Solomon, M. B., Campbell, R. G., Stelle, N. C., and Caperna, T. J. 1989. Effects of exogenous porcine somatotropin administration between 30 and 60 kilograms on longissimus muscle fiber morphology and meat tenderness of pigs grown to 90 kilograms. J. Anim. Sci. 67(Suppl. 1):153. Abstr.

Squire, J. M. 1986. Myosin molecule. pp. 116-146. In Muscle Design, Diversity and Disease. Benjamin/Cummings, Menlo Park, CA.

Stabursvik, E., Fretheim, K., and Froystein, T. 1984. Myosin denaturation in pale soft, and exudative (PSE) porcine muscle tissue as studied by differential scanning calorimetry. J. Sci. Food Agric. 35:240.

Starr, K. and Offer, G. 1978. The interaction of C-protein with heavy meromyosin and subfragment-2. Biochem. J. 171:813.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., and Singleton, R. C. 1974. Sensory evaluation by quantitative descriptive analysis. Food Technol. 28:24.

Suzuki, A., Goll, D. E., Singh, I., Allen, R. E., Robson, R. M., and Stromer, M. H. 1976. Some properties of purified skeletal muscle alpha-actinin. J. Biol. Chem. 251:6860.

Suzuki, K., Tsuji, S., Ishiura, S., Kimura, Y., Kubota, S., and Imahori, K. 1981. Autolysis of calcium-activated neutral protease of chicken skeletal muscle. J. Biochem. 90:1787.

Swatland, H. J. 1985. Optical and electronic methods of measuring pH and other predictors of meat quality in pork carcasses. J. Anim. Sci. 61:887.

Swatland, H. J. 1986. Color measurements on pork and veal carcasses by fiber optic spectrophotometry. Can. Inst. Food Sci. Technol. J. 19:170.

Szczesniak, A. S. 1986. Sensory texture evaluation methodology. Proc. Recip. Meat Conf. 39:86.

Takahashi, K. and Saito, H. 1979. Post-mortem changes in skeletal muscle connectin. J. Biochem. 85:1539.

Talmant, A. and Monin, G. 1983. Evolution of sarcoplasmic protein solubility and of certain enzymatic activities in pork muscle as a function of post mortem conditions and genetic type (Halothane sensitivity). Proc. Eur. Meeting Meat Res. Workers 29:172. Abstr.

Tokuyasu, K. T. and Maher, P. A. 1987. Immunocytochemical studies of cardiac myofibrillogenesis in early chick embryos. I. Presence of immunofluorescent titin spots in premyofibril stages. J. Cell Biol. 105:2781.

Topel, D. G. and Hallberg, J. W. 1985. Stress-susceptibility with particular emphasis on carcass quality and health. EAAP Publication 33:49.

Topel, D. G., Miller, J. A., Berger, P. J., Rust, R. E., Parrish, Jr., F. C., and Ono, K. 1976. Palatability and visual acceptance of dark, normal and pale colored *M. longissimus*. J. Food Sci. 41:628.

Topel, D. G., Staun, H., and Riis, H. M. 1975. Relationships between stress adaptation traits in swine with skeletal muscle characteristics. World Rev. Anim. Prod. 10:52.

Trinick, J. and Lowey, S. 1977. M-protein from chicken pectoralis muscle: Isolation and characterization. J. Mol. Biol. 113:343.

Trinick, J., Knight, P., and Whiting, A. 1984. Purification and properties of native titin. J. Mol. Biol. 180:331.

Vada-Kovacs, M., Csiba, A., Nagy-Nemeth, A., and Saska, J. 1982. Significance of $pH_1$ and early rigor for prediction of pork meat quality. Proc. Eur. Meeting of Meat Res. Workers 34:94.

Valin, C. 1968. Post-mortem changes in myofibrillar protein solubility. J. Food Technol. 3:171.

Wang, K. 1982. Purification of titin and nebulin. Methods in Enzym. 85:264.

Wang, K. and Wright, J. 1988. Architexture of the sarcomere matrix of skeletal muscle: Immunoelectron microscopic evidence that suggests a set of parallel inextensible nebulin filaments anchored at the Z-line. J. Cell Biol. 107:2199.

Wang, K., McClure, J., and Tu, A. 1979. Titin: Major myofibrillar components of striated muscle. Proc. Natl. Acad. Sci. 76:3698.

Wang, K., Ramirez-Mitchell, R., and Palter, D. 1984. Titin is an extraordinarily long, flexible and slender myofibrillar protein. Proc. Natl. Acad. Sci. 81:3685.

Warriss, P. D. 1982. The relationship between $pH_{45}$ and drip in pig muscle. J. Food. Technol. 17:573.

Warriss, P. D. and Brown, S. N. 1987. The relationships between initial pH, reflectance and exudation in pig muscle. Meat Sci. 20:65.

Webb, A. J., Southwood, O. I., Simpson, S. P., and Carden, A. E. 1985. Genetics of porcine stress syndrome. EAAP Publications 33:9.

Wheelock, M. J. 1982. Evidence for two structurally different forms of skeletal muscle $Ca^{2+}$-activated protease. J. Biol. Chem. 257:12471.

Whiting, A., Wardale, J., and Trinick, J. 1989. Does titin regulate the length of muscle thick filaments? J. Mol. Biol. 205:263.

Wismer-Pedersen, J. 1960. Effect of cure on pork with watery structure. I. Binding of salt and water to the meat. Food Res. 25:789.

Yamaguchi, M., Izumimoto, M. Robson, R. M., and Stromer, M. H. 1985. Fine structure of wide and narrow vertebrate muscle Z-lines. A proposed model and computer simulation of Z-line architecture. J. Mol. Biol. 184:626.

Yang, T. S., Hawrysh, Z. J., Price, M. A., and Aherne, F. X. 1984. Identification of PSE in *longissimus* muscle of pigs stunned by captive-bolt. Meat Sci. 10:243.

Yates, L. D. and Greaser, M. L. 1983. Quantitative determination of myosin and actin in rabbit skeletal muscle. J. Mol. Biol. 168:123.

Zeece, M. G., Robson, R. M., Lusby, M. L., and Parrish, Jr., F. C. 1986. Effect of calcium activated protease (CAF) on bovine myofibrils under different conditions of pH and temperature. J. Food Sci. 51:797.

Zot, A. S. and Potter, J. D. 1987. Structural aspects of troponin-tropomyosin regulation of skeletal muscle contraction. Ann. Rev. Biophys. Biophys. Chem. 16:535.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my major professor, Dr. F. C. Parrish, Jr., and Dr. Lauren Christian for their assistance, advice and support throughout my studies at Iowa State University. I would also like to thank Dr. Pat Murphy, Dr. Ken Prusa and Dr. Ted Huaitt for serving on my committee and reviewing this thesis.

I extend special thanks to Kathy Schneider and fellow graduate student Maureen Dolan for their technical assistance, advice and moral support. I especially thank Susan Joens and Angie Nissen for their efforts with my research.

My deepest thanks go to my sister, Gail, and my parents, Vernon and Wilma, for their encouragement and patience.